



US009353116B2

(12) **United States Patent**  
**Garske et al.**(10) **Patent No.:** **US 9,353,116 B2**  
(45) **Date of Patent:** **May 31, 2016**(54) **METHODS AND COMPOSITIONS FOR KINASE INHIBITION**(71) Applicant: **THE REGENTS OF THE UNIVERSITY OF CALIFORNIA,**  
Oakland, CA (US)(72) Inventors: **Adam L. Garske**, San Francisco, CA (US); **Kevan M. Shokat**, San Francisco, CA (US)(73) Assignee: **The Regents of the University of California**, Oakland, CA (US)

( \* ) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **13/690,785**(22) Filed: **Nov. 30, 2012**(65) **Prior Publication Data**

US 2013/0137708 A1 May 30, 2013

**Related U.S. Application Data**

(63) Continuation of application No. PCT/US2011/039347, filed on Jun. 6, 2011.

(60) Provisional application No. 61/351,663, filed on Jun. 4, 2010.

(51) **Int. Cl.****C12N 9/12** (2006.01)**C12N 9/99** (2006.01)**C07D 487/04** (2006.01)**C07D 239/94** (2006.01)**C07D 403/12** (2006.01)**C07D 409/12** (2006.01)**A01N 43/90** (2006.01)**A61K 31/519** (2006.01)(52) **U.S. Cl.**CPC ..... **C07D 487/04** (2013.01); **C07D 239/94** (2013.01); **C07D 403/12** (2013.01); **C07D 409/12** (2013.01); **C12N 9/12** (2013.01)(58) **Field of Classification Search**

None

See application file for complete search history.

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## (57)

**ABSTRACT**

The present invention sets forth a new chemical genetic approach for engineering kinase enzymes with a cysteine gatekeeper residue as well as for developing electrophilic inhibitors thereto. The present invention also provides a Src proto-oncogenic tyrosine kinase with a cysteine gatekeeper that recapitulates wild type activity and can be irreversibly inhibited both in vitro and in cells. The present invention also provides methods and compositions for modulating kinases and for treating kinase-associate diseases.

**12 Claims, 22 Drawing Sheets**

FIG. 1

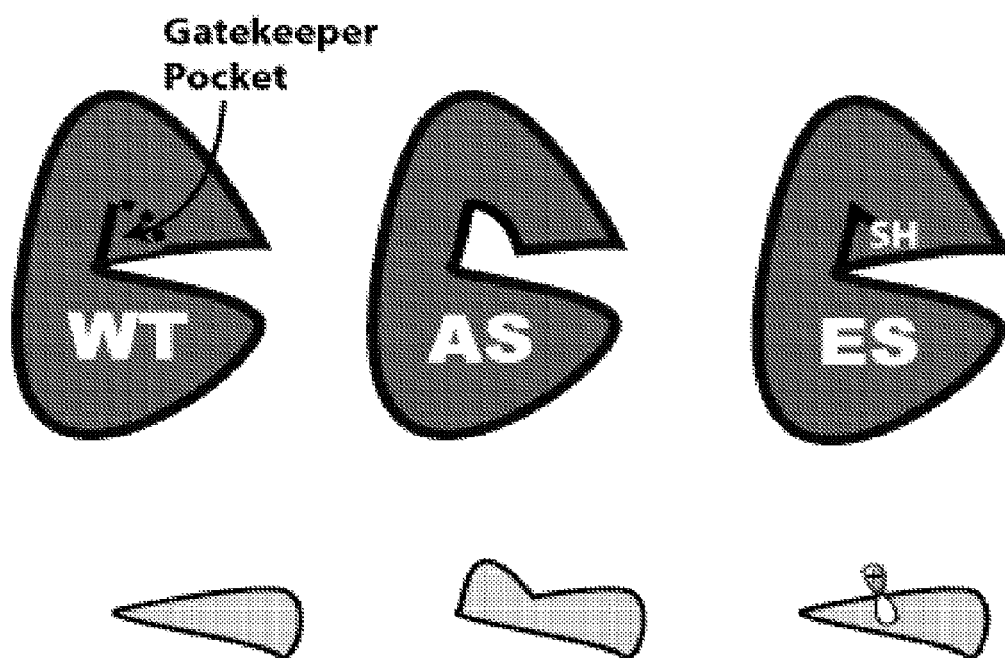


FIG. 2

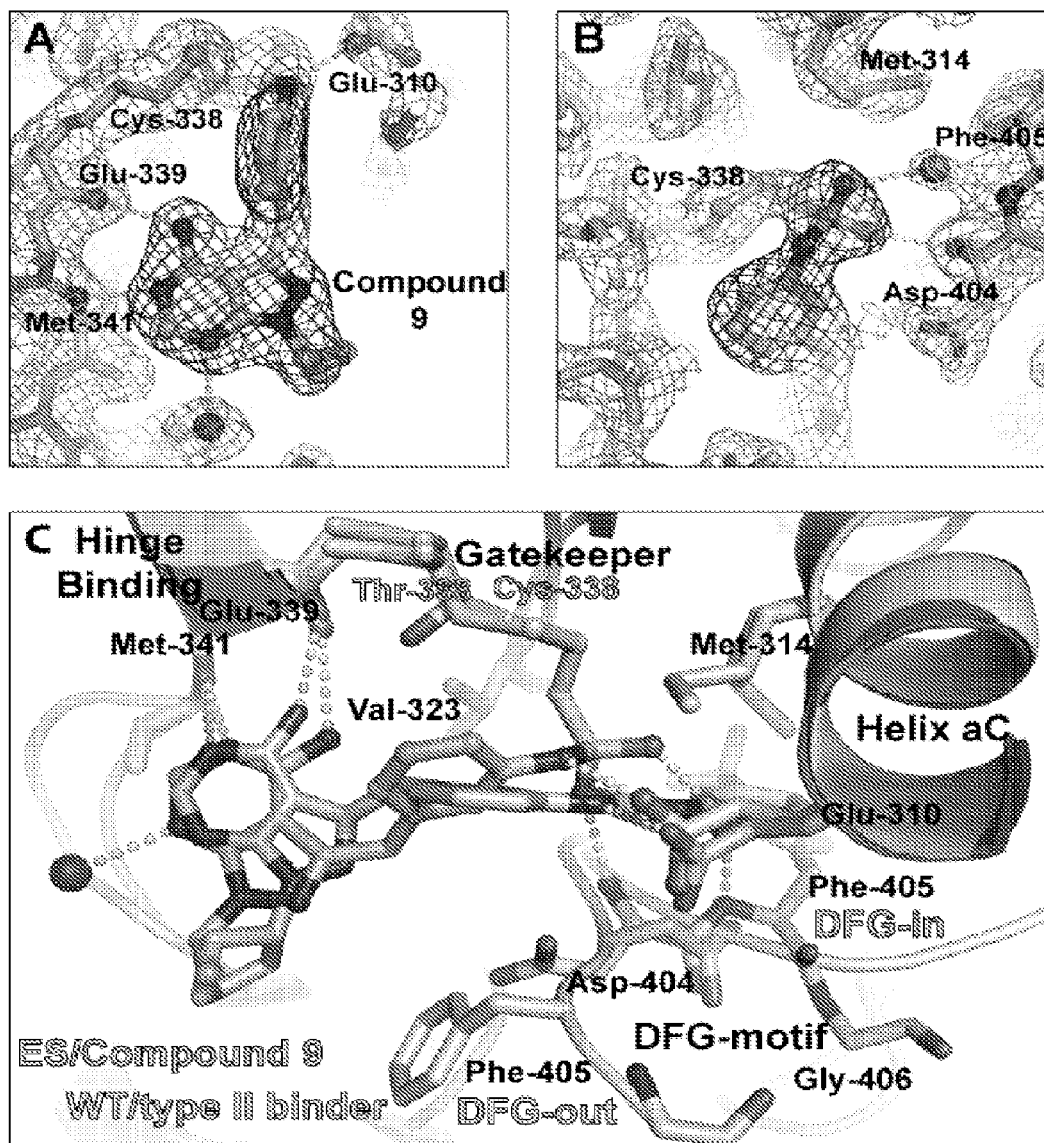
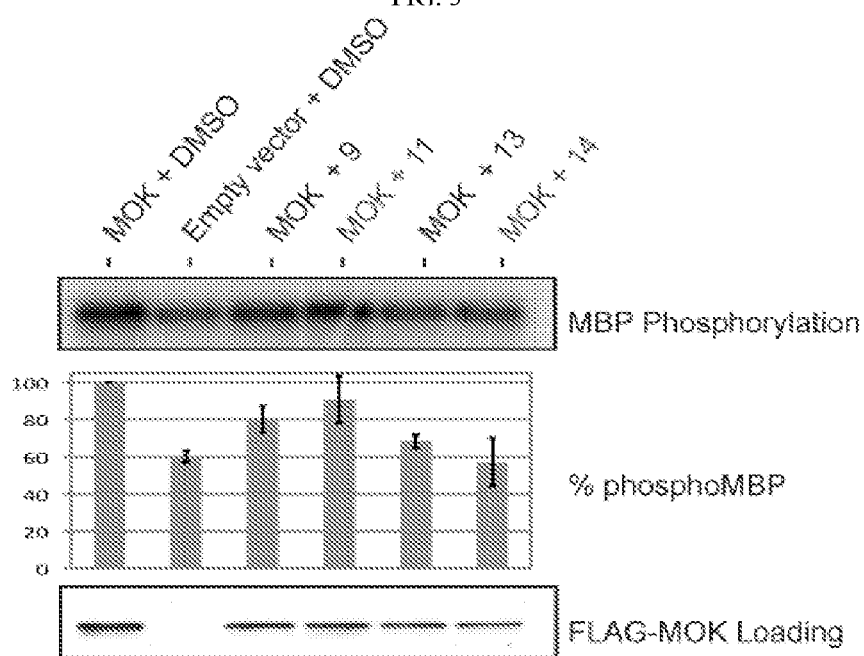


FIG. 3



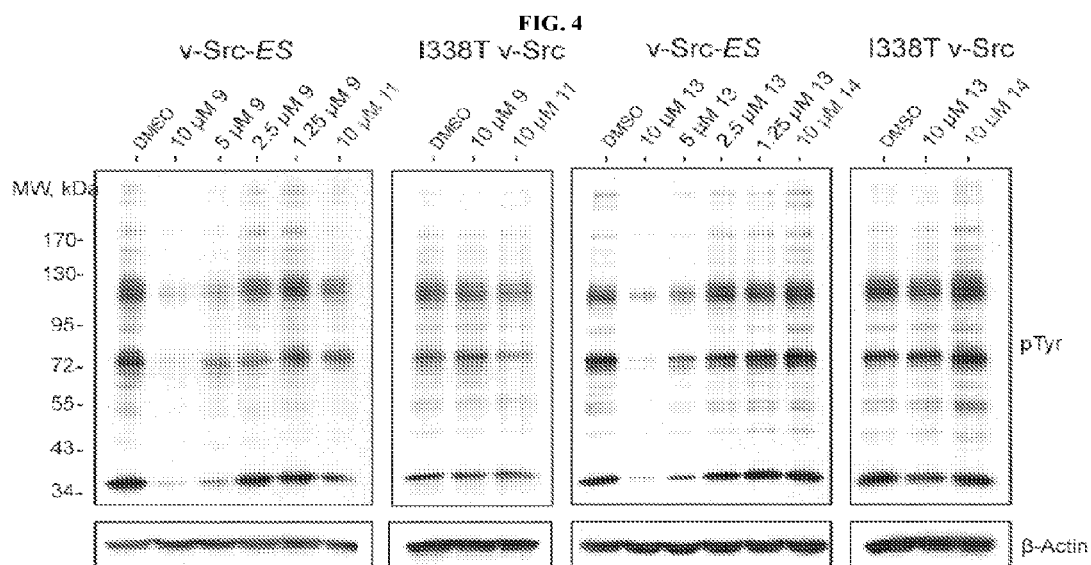


FIG. 5

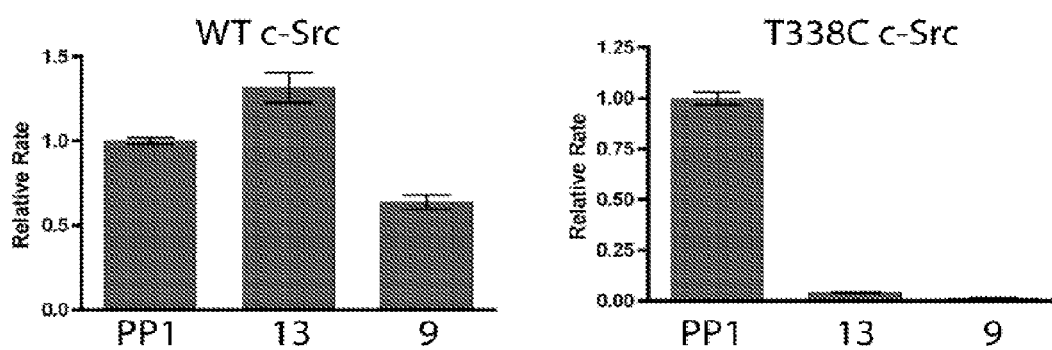


FIG. 6

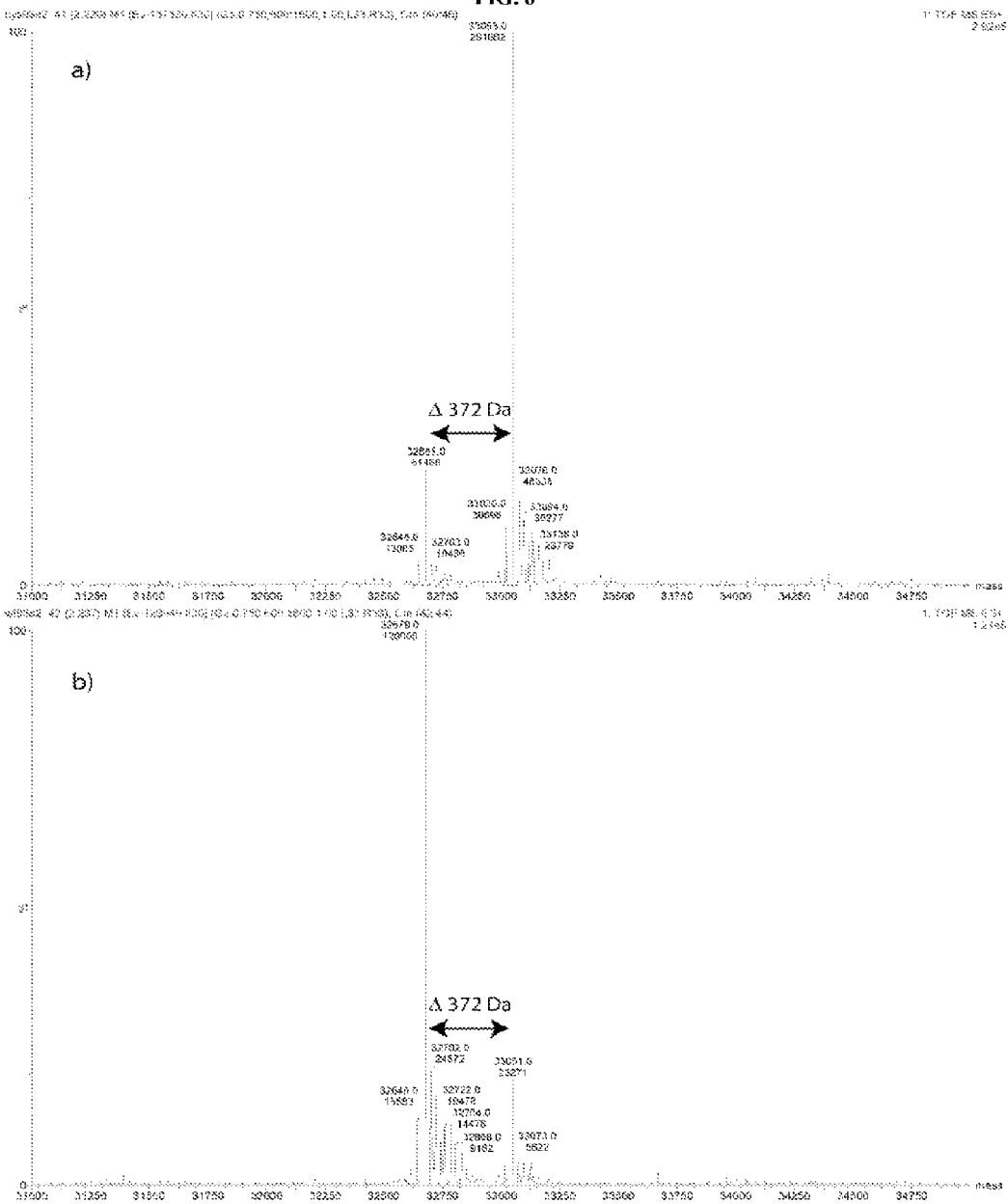


FIG. 7

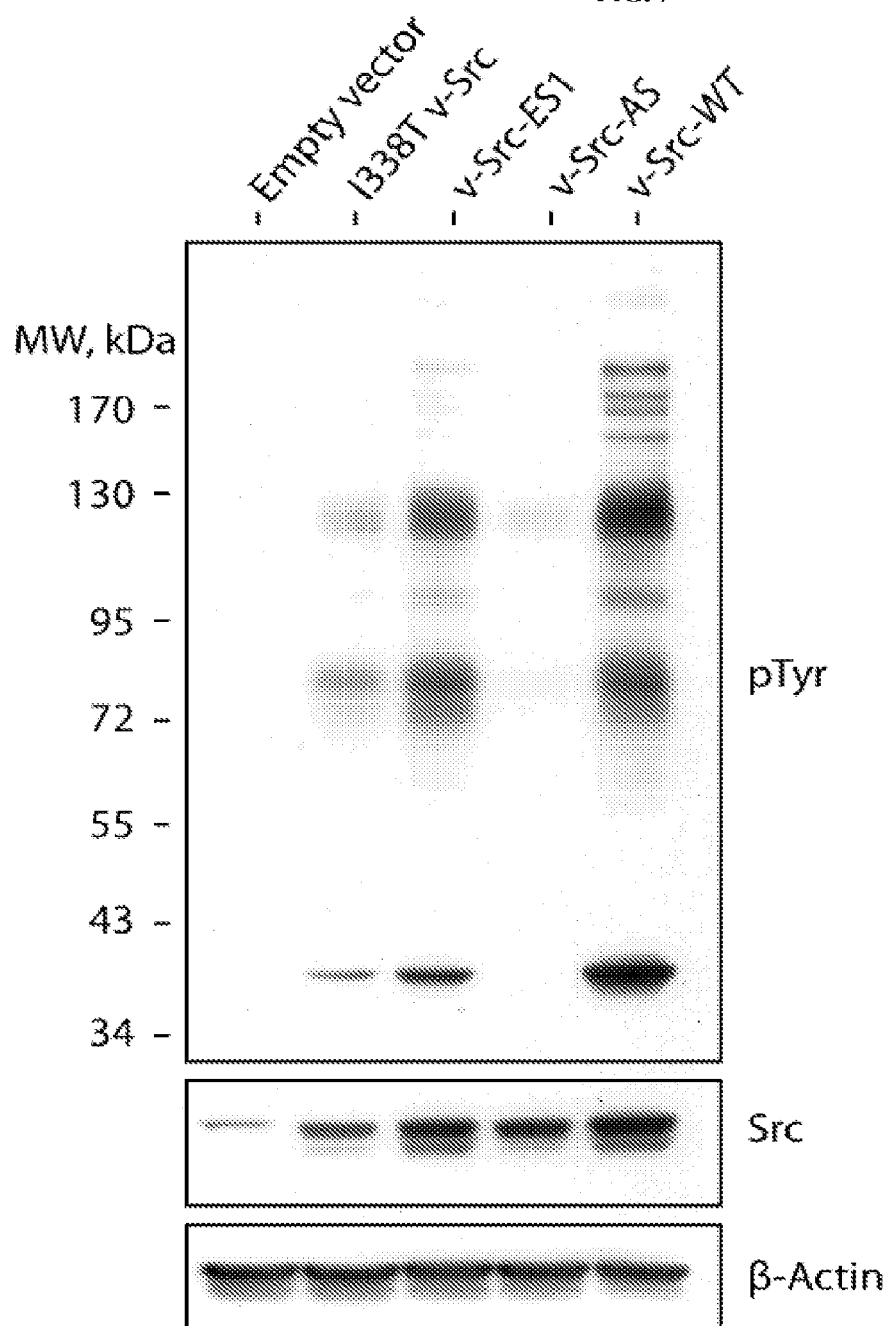


FIG. 8

Thu May 26, 2011 14:21 PDT

AG\_144492-2\_cSRCF-T338C-A\_t/promoterB06.seq

/Users/algarske/Documents/Sequencing/cSRC/AG\_144492-2\_cSRcf-T338C-A\_t7promoterB06.seq

From 1 to 918.

Translation 305 a.a. MW=35116.20999999997

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F L K G E M G K Y L R L P Q L V D M A A  
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Q I A S G M A Y V E R M N Y V H R D L R  
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A A N I L V G E N L V C K V A D F G L A  
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R L I E D N E Y T A R Q G A K F P I K W  
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T A P E A A L Y G R F T I K S D V W S F  
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E S L H D L M C Q C W R K D P E E R P T  
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F E Y L Q A F L E D Y F T S T E P Q Y Q  
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P G E N L \*  
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FIG. 9

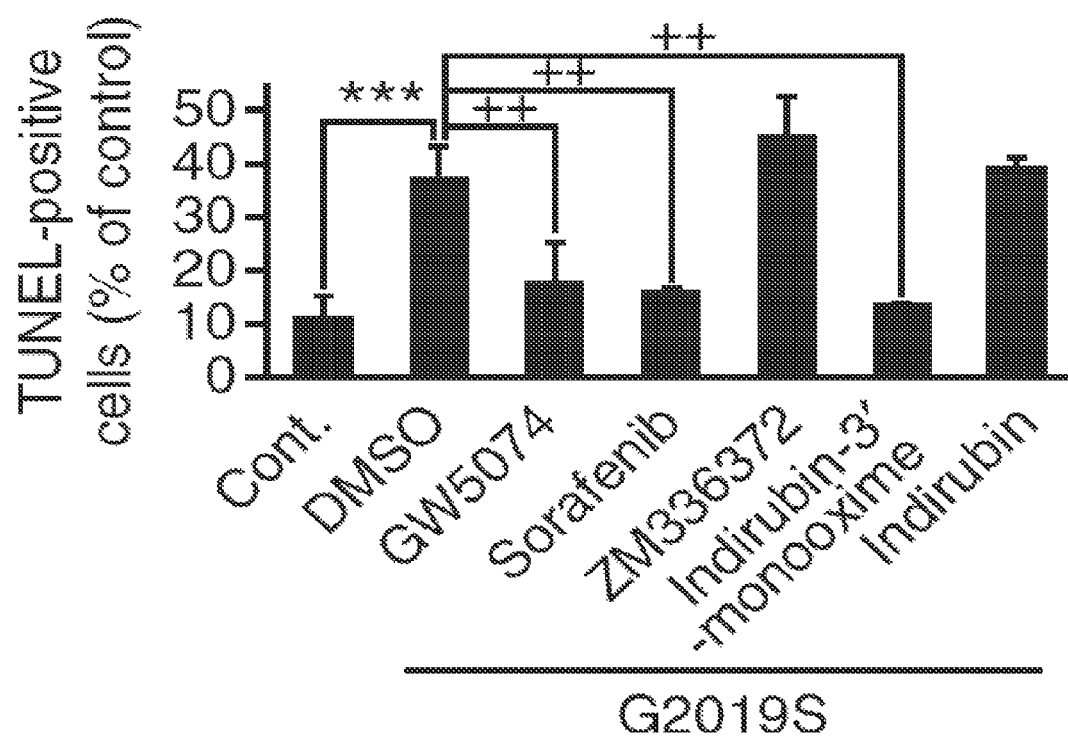


FIG. 10

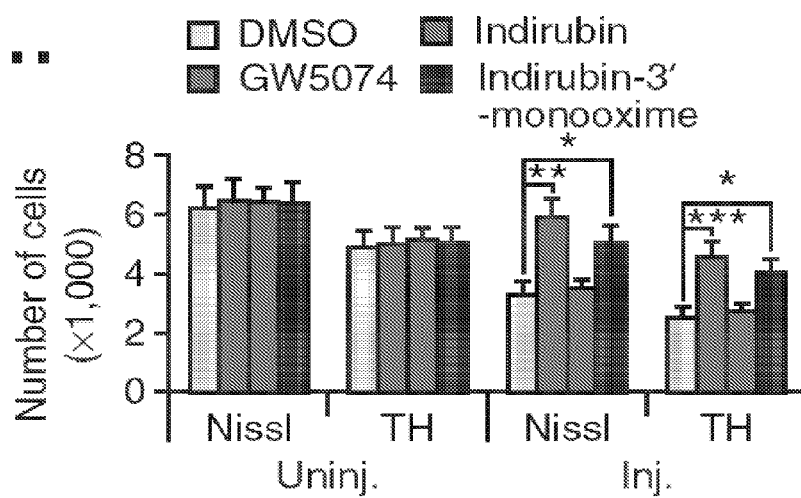


FIG. 11

	3-vs-Q
BTK	59
CHEK2 (CHK2)	53
EGFR (ErbB1)	84
EGFR (ErbB1) L858R	69
EGFR (ErbB1) L861Q	76
EGFR (ErbB1) T790M L858R	41
ERBB2 (HER2)	66
ERBB4 (HER4)	80
FLT3 D835Y	99
GRK5	49
LRRK2	75
LRRK2 G2019S	91
PDGFRA V561D	45
PIK3C2B (PI3K-C2 beta)	65
RPS6KA6 (RSK4)	49
SRMS (Srm)	71
TXK	78

FIG. 12	
WT LRRK2	G2019S LRRK2
156 nM	33 nM
>1000 nM	>1000 nM
>1000 nM	>1000 nM
>1000 nM	>1000 nM

FIG. 13  
LRRK2 WT

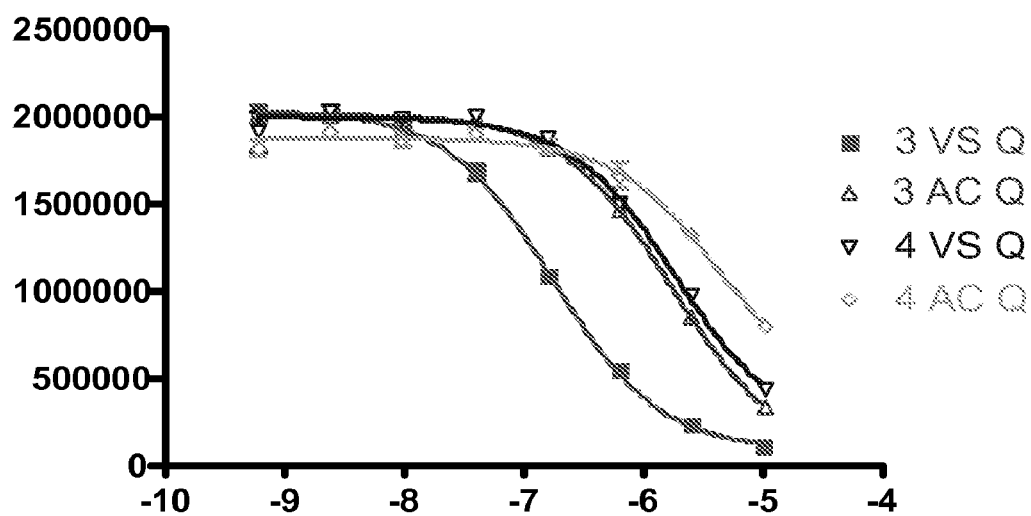


FIG. 14  
LRRK2 GS

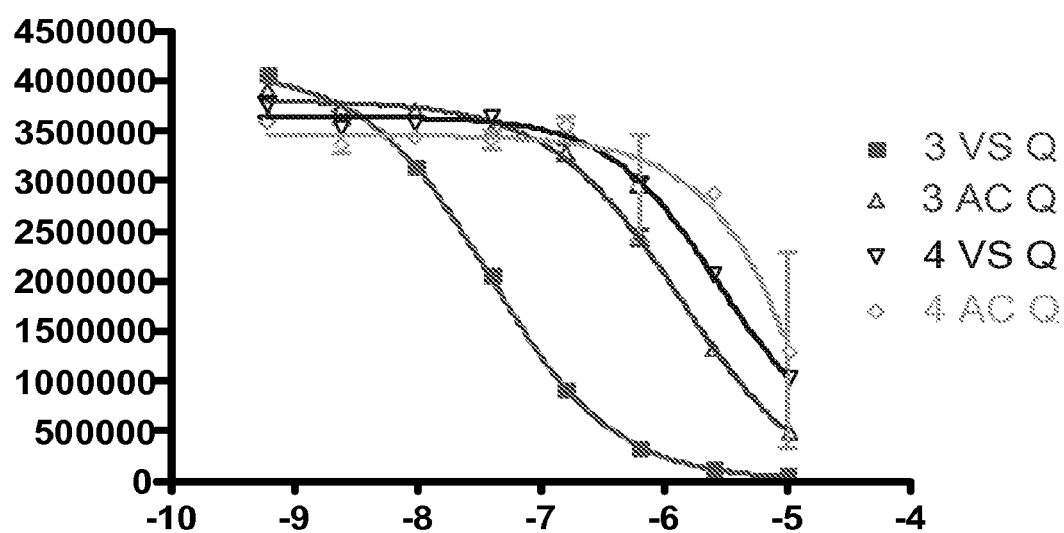


FIG. 15

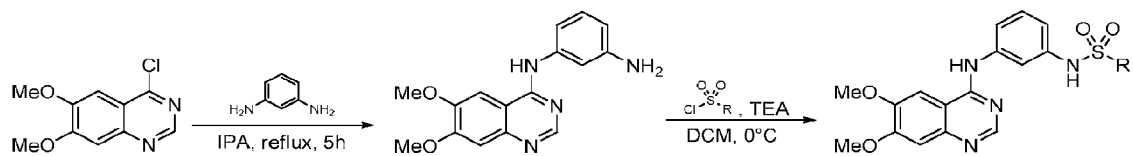


FIG. 16




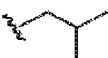


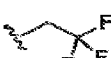

R=	WT LRRK2 IC <sub>50</sub>	G2019SLRRK2 IC <sub>50</sub>
 (3-vs-Q)	213 nM	45.5 nM
 Me	353 nM	100 nM
	334 nM	121 nM
	241 nM	90.1 nM
	186 nM	46.9 nM
	329 nM	57.7nM
	380 nM	274 nM
	334 nM	121 nM

FIG. 17

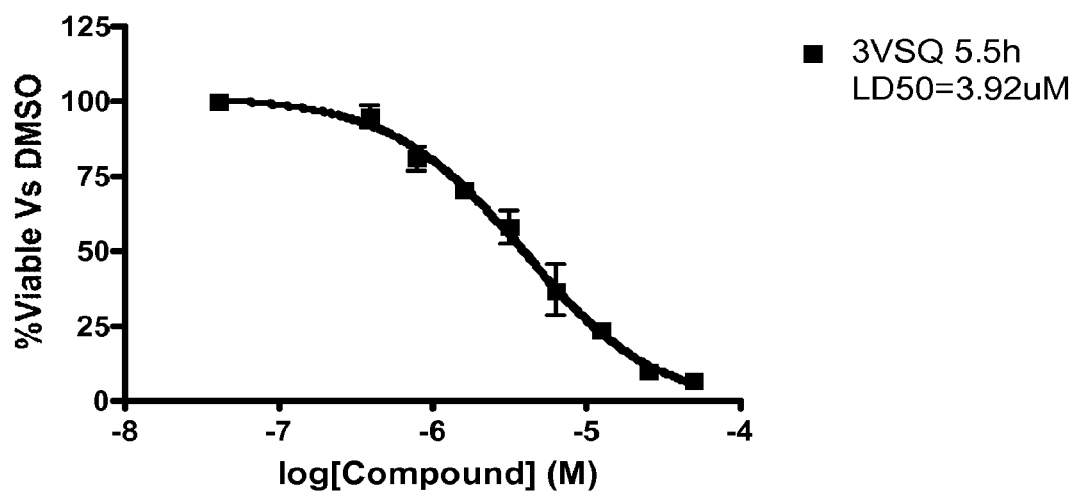
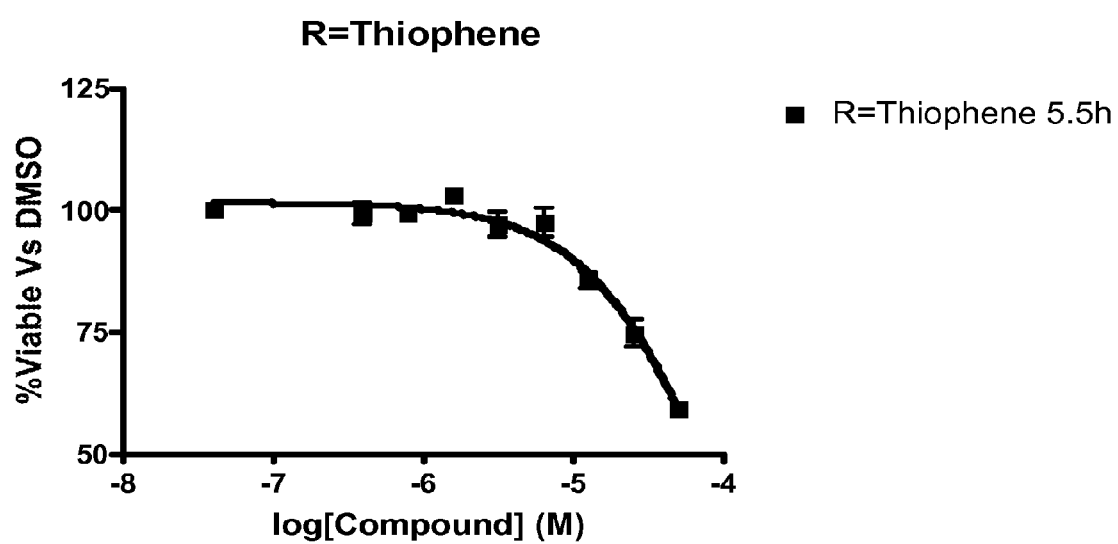
**3VSQ**

FIG. 18



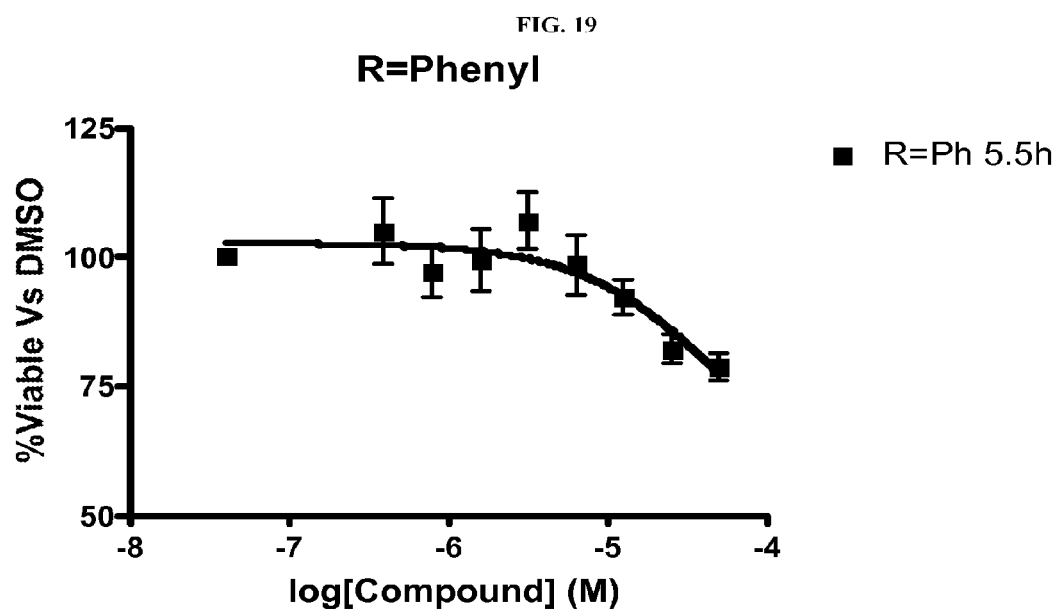


FIG. 20

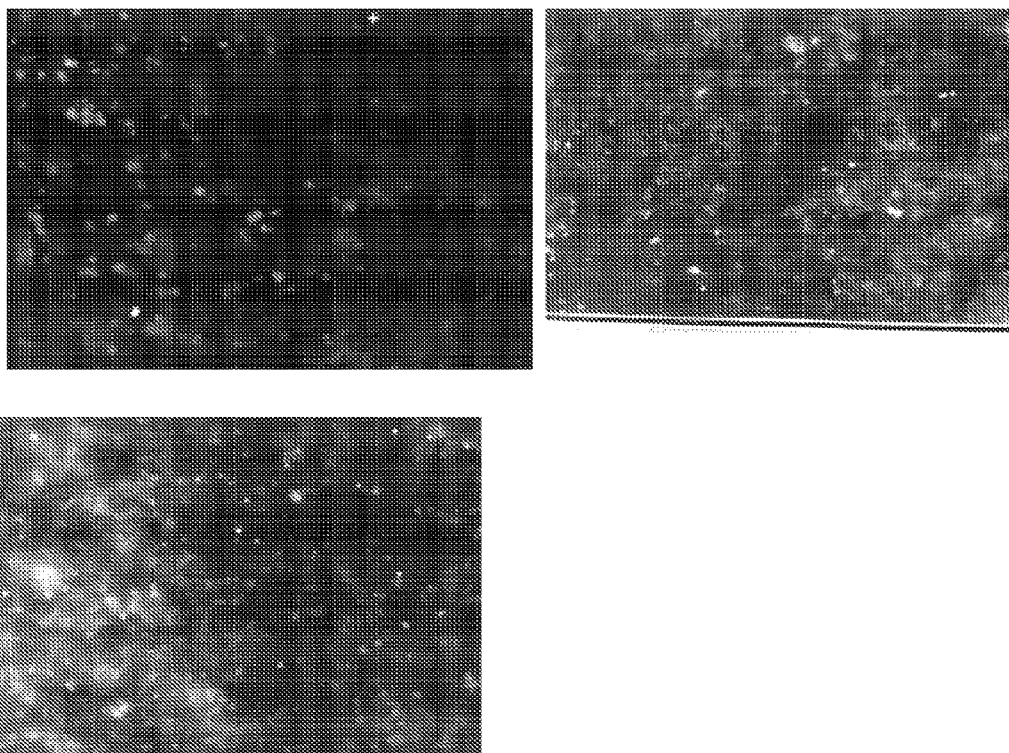


FIG. 21

### LRRK2 G2019S in-vitro Kinase Assay

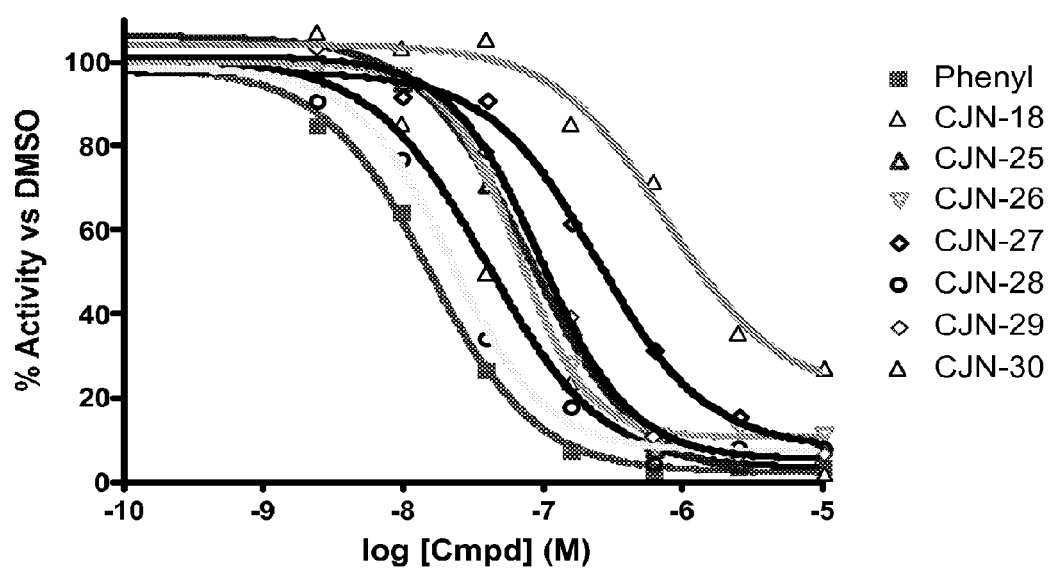
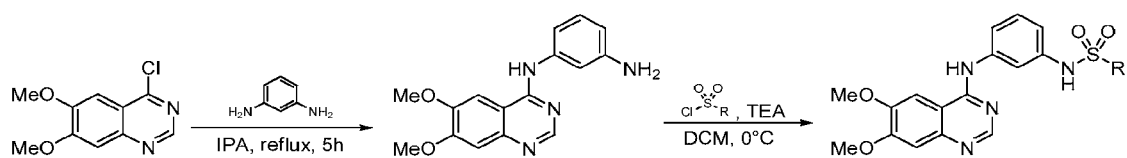


FIG. 22



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# METHODS AND COMPOSITIONS FOR KINASE INHIBITION

## CROSS-REFERENCES TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Patent Application 61/351,663, filed Jun. 4, 2010, and International Patent Application PCT/US2011/039347, filed Jun. 6, 2011, which are hereby incorporated by reference in their entirety for all purposes.

## STATEMENT AS TO RIGHTS TO INVENTIONS MADE UNDER FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT REFERENCE TO A "SEQUENCE LISTING," A TABLE, OR A COMPUTER PROGRAM LISTING APPENDIX SUBMITTED AS AN ASCII TEXT FILE

The Sequence Listing written in file -122-1.TXT, created on Jan. 23, 2013, 229,376 bytes, machine format IBM-PC, MS-Windows operating system, is hereby incorporated by reference in its entirety for all purposes.

This invention was made with Government support under Grant Nos. 5F32CA138103-2 and 1R01EB001987-16, awarded by the National Institutes of Health. The Government has certain rights in this invention.

## BACKGROUND OF THE INVENTION

Kinases, which constitute a large family of enzymes (>500 in humans), catalyze the transfer of the  $\gamma$ -phosphate of ATP to protein substrates. Reversible phosphorylation plays a paramount role in cell signaling processes and is regulated by kinases and phosphatases. Accordingly, kinases are critical mediators of a myriad of signal transduction processes. Aberrant kinase activity is linked to cancer as well as metabolic, immunological, and nervous system disorders. As a result, kinases have emerged as an important class of drug targets for human disease. However, due to the conserved nature of the active sites of the protein kinase family, it is difficult to obtain selective inhibitors for any one kinase.

There are at least 518 kinases, such as those which catalyze the transfer of the gamma phosphate of ATP to protein and small molecule substrates and are involved in cell signaling processes. Small molecules provide a means for delineating kinase signaling because they are fast acting and dosable. However, because all kinase active sites recognize ATP, it is difficult to develop selective ATP-competitive inhibitors. Several years ago, a chemical genetic strategy for selective kinase inhibition was developed with reversible inhibitors (U.S. Patent Publication No. 2009/0221614). The chemical genetic strategy involves the engineered mutation of a conserved bulky residue in the kinase active site known as "the gatekeeper" to a small residue such as glycine or alanine (See Bishop A C, et al. (1998) Design of allele-specific inhibitors to probe protein kinase signaling. *Curr Biol* 8(5):257-266; and Bishop A C, et al. (2000) A chemical switch for inhibitor-sensitive alleles of any protein kinase. *Nature* 407(6802):395-401). The engineered active site can then accommodate an inhibitor capable of occupying the newly formed binding pocket. While this strategy has utility, mutation of the gatekeeper residue to a small amino acid may impair the activity of the kinase and the selective inhibition can only be applied to one kinase at a time. In addition, it is sometimes not possible to achieve the desired potency.

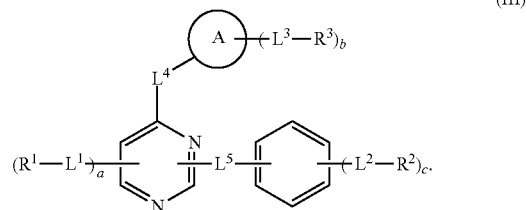
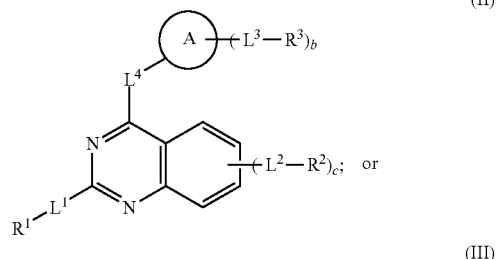
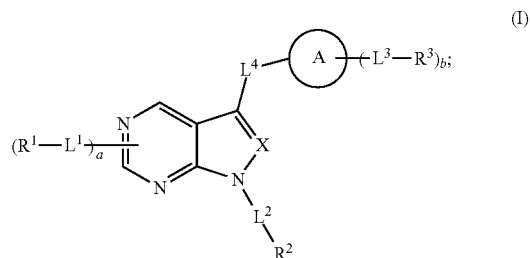
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It is known in the field that mutations in Leucine-Rich Repeat Kinase 2 (Lrrk-2) can lead to Parkinsons Disease. Also, it is thought that Parkinson's Disease (PD) is caused by uncontrolled apoptosis of dopaminergic neurons. Because inhibition of Lrrk-2 kinase activity can inhibit the apoptotic effects, there is a need to develop inhibitors for Lrrk-2 to provide treatments for Parkinson's Disease.

As such, there is a need in the field to develop kinase gatekeeper residue mutations which do not diminish kinase activity or ATP affinity as well as small molecules which inhibit these kinases. There is also a need to develop effective Lrrk-2 inhig. Surprisingly, the present invention solves these as well as other problems in the field.

## BRIEF SUMMARY OF THE INVENTION

In one aspect, the present invention provides a compound having the formula:



X is  $=N-$  or  $=C(L^6-R^6)-$ . Ring A is, in each instance, independently selected from cycloalkyl, heterocycloalkyl, aryl, or heteroaryl;  $L^1$ ,  $L^2$ ,  $L^3$ ,  $L^4$ ,  $L^5$ , and  $L^6$  are, in each instance, independently selected from a bond,  $-C(O)-$ ,  $-C(O)N(R^7)-$ ,  $-C(O)O-$ ,  $-S(O)_g-$ ,  $-S(O)_2N(R^7)-$ ,  $-O-$ ,  $-N(R^7)-$ ,  $-N(R^7)C(O)N(R^8)-$ , substituted or unsubstituted alkylene, substituted or unsubstituted heteroalkylene, substituted or unsubstituted cycloalkylene, substituted or unsubstituted heterocycloalkylene, substituted or unsubstituted arylene, or substituted or unsubstituted heteroarylene, wherein g is an integer from 0 to 2;  $R^1$ ,  $R^2$ ,  $R^3$ ,  $R^4$ ,  $R^5$ ,  $R^6$ ,  $R^7$ , and  $R^8$  are, in each instance, independently selected from hydrogen, halogen,  $-CN$ ,  $-OH$ ,  $-NH_2$ ,  $-COOH$ ,  $-CONH_2$ ,  $-NO_2$ ,  $-SH$ ,  $-SO_2Cl$ ,  $-SO_3H$ ,  $-SO_4H$ ,  $-SO_2NH_2$ , substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocy-

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cloalkyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl; a is an integer from 0 to 2; b is an integer from 0 to 5; and c is an integer from 0 to 4.

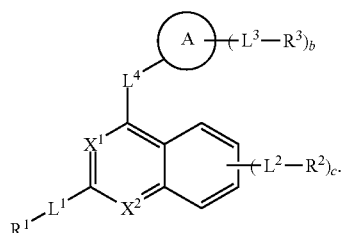
In a second aspect, the present invention provides a recombinant kinase comprising a cysteine substitution at a gatekeeper amino acid position.

In a third aspect, the present invention provides a co-crystal comprising a recombinant kinase and a compound of provided herein (e.g. formula I, II, or III).

In a fourth aspect, the present invention provides an isolated nucleic acid comprising a polynucleotide sequence encoding a recombinant kinase provided herein.

In a fifth aspect, the present invention provides a method of inhibiting a recombinant kinase provided herein, comprising contacting the recombinant kinase with an effective amount of an inhibitor provided herein, thereby inhibiting the recombinant kinase.

In a sixth aspect, the present invention provides a compound having the formula:



$X^1$  and  $X^2$  are, in each instance, independently  $=N-$  or  $=C(-L^6-R^6)-$ . Ring A is as defined above.  $R^1$ ,  $R^2$ , and  $R^3$  are as defined above.  $L^1$ ,  $L^2$ , and  $L^3$  are as defined above. The variables b and c are as defined above.

In a seventh aspect, the present invention provides a method of inhibiting a Lrrk-2 kinase, the method comprising contacting the Lrrk-2 kinase with an effective amount of a Lrrk-2 inhibitor, thereby inhibiting the Lrrk-2 kinase.

In an eighth aspect, the present invention provides a method of forming a recombinant kinase, comprising transforming a cell with a nucleic acid as set forth herein, thereby forming a recombinant kinase as set forth herein.

In a ninth aspect, the present invention provides a method of treating a kinase-associated disease or condition, in a patient in need thereof, said method comprising administering to said patient a therapeutically effective amount of a compound of the present invention, thereby treating a kinase-associated disease or condition.

In a tenth aspect, the present invention provides a method of treating a Lrrk-2-associated disease or condition, in a patient in need thereof, said method comprising administering to said patient a therapeutically effective amount of a compound of the present invention, thereby treating a Lrrk-2-associated disease or condition.

In an eleventh aspect, the present invention provides a kit comprising, a recombinant kinase or a nucleic acid provided herein; and instructions for using the kit.

#### BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows a schematic of the chemical genetic strategies for inhibiting protein kinases. Kinases are depicted on top, e.g. WT, AS, and ES, and inhibitors types are represented on the bottom. Wild type (WT) kinases generally harbor hydrophobic gatekeeper residues and may not be inhibited selec-

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tively. An analog-sensitive (AS) protein kinase has an engineered glycine or alanine gatekeeper and may be selectively inhibited by a bulky inhibitor. An electrophile-sensitive (ES) protein kinase contains an engineered cysteine gatekeeper and may be selectively inhibited by an electrophilic inhibitor.

FIG. 2 shows the crystal structure of compound 9 bound covalently to c-Src-ES1. The experimental electron density of c-Src-ES1 at 2.20 Å resolution is shown ( $2F_o - F_c$  map at 1σ). (A) The pyrazolopyrimidine portion of compound 9 (green) interacts with the hinge region of c-Src (Met-341 and Glu-339), while the sulfonamide group makes a hydrogen bonds with Glu-310 of the αC helix (B) Electron density reveals a covalent linkage between Cys-338 and compound 9. The oxygen atoms of the sulfonamide interact with the backbone of Asp-404 and via a water molecule with Phe-405, both of which are part of the DFG-motif of the kinase (C) Comparison of structural features of compound 9 bound to c-Src-ES1 and a known pyrazolopyrimidine compound bound to WT c-Src. Both compounds engage the hinge region in a similar fashion and bind the αC helix in the “in” conformation. Furthermore, both compounds participate in hydrogen bonding interactions with Glu-310 and backbone amides of the DFG-motif. However while the known pyrazolopyrimidine compound binds in the “DFG-out” conformation, compound 9 engages the “DFG-in” orientation. The sulfhydryl of the Cys-338 points in the opposite direction relative to the hydroxyl group of Thr-338 in order to facilitate a covalent bond with compound 9.

FIG. 3 shows an assay for MOK inhibition by cysteine gatekeeper-targeting compounds. (top) FLAG-MOK expressed in COST cells was immunoprecipitated and assayed in vitro with a myelin basic protein (MBP) substrate and inhibitors at a concentration of 1 μM. Autoradiography is shown. (center) Quantification of the percent MBP phosphorylated from three independent experiments with associated standard errors. All values are normalized relative to the MOK+DMSO lane. (bottom) Western blot of loading controls for FLAG-MOK are shown.

FIG. 4 shows a cellular dose response analysis for inhibition of v-Src-ES1 (I338C) with electrophilic inhibitors. Cells transfected NIH-3T3 with either v-Src-ES1 or I338T v-Src were treated with electrophilic inhibitors or non-reactive analogs for one hour (see the far right column of each run, e.g. 10 μM 11; 10 μM 11; 10 μM 14; 10 μM 14). Kinase activity was monitored by blotting for global phosphotyrosine levels. Actin blots were included to control for protein content.

FIG. 5 shows relative rates of wild type c-Src and T338C c-Src following treatment with PP1, 13 or 9 and purification by gel filtration. Assay was done in triplicate, and average values with standard errors are given.

FIG. 6 shows ESI-MS/TOF mass spectral analysis of covalent labeling of T338C c-Src and WT c-Src with compound 9. T338C c-Src (a) or WT c-Src (b) (15 μM) was incubated with two equivalents of compound 9 and analyzed by full-protein mass spectrometry after 5 minutes of reaction. A 372 Da mass change occurs upon covalent labeling. Deconvoluted mass spectra are shown.

FIG. 7 shows analysis of the activity of v-Src gatekeeper variants in cells by Western blot. NIH-3T3 cells lines were infected with several v-Src gatekeeper variants. The kinase activity of the variants was analyzed by blotting for global phosphotyrosine levels (pTyr). The Src and actin blots account for Src expression levels and total protein content, respectively.

FIG. 8 shows the amino acid sequence (SEQ ID NO: 2) of Src and also the nucleic acid sequence (SEQ ID NO: 1) encoding therefor.

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FIG. 9 shows inhibition of Lrrk-2 kinase activity.

FIG. 10 shows inhibition of Lrrk-2 kinase activity.

FIG. 11 shows selectivity of compound 19 (3-vs-Q) in the Invitrogen SelectScreen Kinase Assay.

FIG. 12 shows SAR analysis and inhibition as dependent on a vinylsulfonamide in the 3 position.

FIG. 13 shows SAR analysis and inhibition as dependent on a vinylsulfonamide in the 3 position.

FIG. 14 shows SAR analysis and inhibition as dependent on a vinylsulfonamide in the 3 position.

FIG. 15 shows the synthesis of compounds suitable for use with the present invention.

FIG. 16 shows in vitro kinase assay data for wild type and G2019S Lrrk-2.

FIG. 17 shows toxicity profiles. LD50 of compound 19 (3-vs-Q) LD50=3.92  $\mu$ M; All other compounds  $\geq$ 50  $\mu$ M.

FIG. 18 shows toxicity profiles. LD50 of compound 19 (3-vs-Q) LD50=3.92  $\mu$ M; All other compounds  $\geq$ 50  $\mu$ M.

FIG. 19 shows toxicity profiles. LD50 of compound 19 (3-vs-Q) LD50=3.92  $\mu$ M; All other compounds  $\geq$ 50  $\mu$ M.

FIG. 20 shows immunocytochemistry. Top left: Staruasporeine, TUNEL stain; Top Right: G2019S mutant, -drug; Bottom: G2019S mutant, +Th.

FIG. 21 shows assay data.

FIG. 22 shows a synthesis of compounds suitable for use with the present invention.

## DETAILED DESCRIPTION OF THE INVENTION

### I. General

Provided herein, inter alia, are methods and compositions for imparting to a kinase the capability of being inhibited by a heterocyclic compound e.g., a cysteine substituted kinase having a gatekeeper amino acid residue within an ATP binding site of a kinase replaced with a cysteine residue. Also provided are methods and compositions for inhibiting a kinase with a heterocyclic compound. Furthermore, methods and compositions are provided for determining a biological activity of a kinase and treating kinase-associate diseases. In addition, methods and compositions are provided for inhibiting a Lrrk-2 kinase.

### II. Definitions

The term "alkyl," by itself or as part of another substituent, means, unless otherwise stated, a straight (i.e. unbranched) or branched chain, or combination thereof, which may be fully saturated, mono- or polyunsaturated and can include di- and multivalent radicals, having the number of carbon atoms designated (i.e. C<sub>1</sub>-C<sub>10</sub> means one to ten carbons). Examples of saturated hydrocarbon radicals include, but are not limited to, groups such as methyl, ethyl, n-propyl, isopropyl, n-butyl, t-butyl, isobutyl, sec-butyl, homologs and isomers of, for example, n-pentyl, n-hexyl, n-heptyl, n-octyl, and the like. An unsaturated alkyl group is one having one or more double bonds or triple bonds. Examples of unsaturated alkyl groups include, but are not limited to, vinyl, 2-propenyl, crotyl, 2-isopentenyl, 2-(butadienyl), 2,4-pentadienyl, 3-(1,4-pentadienyl), ethynyl, 1- and 3-propynyl, 3-butylnyl, and the higher homologs and isomers.

The term "alkylene" by itself or as part of another substituent means a divalent radical derived from an alkyl, as exemplified, but not limited, by —CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>—, —CH<sub>2</sub>CH=CH—CH<sub>2</sub>—, —CH<sub>2</sub>C≡CCH<sub>2</sub>—, —CH<sub>2</sub>CH<sub>2</sub>CH(CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>)CH<sub>2</sub>—. Typically, an alkyl (or alkylene) group will have from 1 to 24 carbon atoms, with those groups having 10 or fewer carbon atoms being preferred

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in the present invention. A "lower alkyl" or "lower alkylene" is a shorter chain alkyl or alkylene group, generally having eight or fewer carbon atoms.

The term "heteroalkyl," by itself or in combination with another term, means, unless otherwise stated, a stable straight or branched chain, or combinations thereof, consisting of at least one carbon atoms and at least one heteroatom selected from the group consisting of O, N, P, Si and S, and wherein the nitrogen, phosphorus, and sulfur atoms may optionally be oxidized and the nitrogen heteroatom may optionally be quaternized. The heteroatom(s) O, N, P and S and Si may be placed at any interior position of the heteroalkyl group or at the position at which alkyl group is attached to the remainder of the molecule. Examples include, but are not limited to,

—CH<sub>2</sub>—CH<sub>2</sub>—O—CH<sub>3</sub>, —CH<sub>2</sub>—CH<sub>2</sub>—NH—CH<sub>3</sub>, —CH<sub>2</sub>—CH<sub>2</sub>—N(CH<sub>3</sub>)—CH<sub>3</sub>, —CH<sub>2</sub>—S—CH<sub>2</sub>—CH<sub>3</sub>, —CH<sub>2</sub>—CH<sub>2</sub>—, —S(O)—CH<sub>3</sub>, —CH<sub>2</sub>—CH<sub>2</sub>—S(O)<sub>2</sub>—CH<sub>3</sub>, —CH=CH—O—CH<sub>3</sub>, —Si(CH<sub>3</sub>)<sub>3</sub>, —CH<sub>2</sub>—CH=N—OCH<sub>3</sub>, —CH=CH—N(CH<sub>3</sub>)—CH<sub>3</sub>, O—CH<sub>3</sub>, —O—CH<sub>2</sub>—CH<sub>3</sub>, and —CN. Up to two or three heteroatoms may be consecutive, such as, for example, —CH<sub>2</sub>—NH—OCH<sub>3</sub> and —CH<sub>2</sub>—O—Si(CH<sub>3</sub>)<sub>3</sub>. Similarly, the term "heteroalkylene" by itself or as part of another substituent means a divalent radical derived from heteroalkyl, as exemplified, but not limited by, —CH<sub>2</sub>—CH<sub>2</sub>—S—CH<sub>2</sub>—CH<sub>2</sub>— and —CH<sub>2</sub>—S—CH<sub>2</sub>—CH<sub>2</sub>—NH—CH<sub>2</sub>—.

For heteroalkylene groups, heteroatoms can also occupy either or both of the chain termini (e.g., alkyleneoxo, alkylenedioxo, alkyleneamino, alkylenediamino, and the like). Still further, for alkylene and heteroalkylene linking groups, no orientation of the linking group is implied by the direction in which the formula of the linking group is written. For example, the formula —C(O)OR'— represents both —C(O)OR'— and —R'OC(O)—. As described above, heteroalkyl groups, as used herein, include those groups that are attached to the remainder of the molecule through a heteroatom, such as —C(O)R', —C(O)NR', —NR'R", —OR', —SR', and/or —SO<sub>2</sub>R'. Where "heteroalkyl" is recited, followed by recitations of specific heteroalkyl groups, such as —NR'R" or the like, it will be understood that the terms heteroalkyl and —NR'R" are not redundant or mutually exclusive. Rather, the specific heteroalkyl groups are recited to add clarity. Thus, the term "heteroalkyl" should not be interpreted herein as excluding specific heteroalkyl groups, such as —NR'R" or the like.

The terms "cycloalkyl" and "heterocycloalkyl", by themselves or in combination with other terms, represent, unless otherwise stated, cyclic versions of "alkyl" and "heteroalkyl", respectively. Additionally, for heterocycloalkyl, a heteroatom can occupy the position at which the heterocycle is attached to the remainder of the molecule. Examples of cycloalkyl include, but are not limited to, cyclopentyl, cyclohexyl, 1-cyclohexenyl, 3-cyclohexenyl, cycloheptyl, and the like. Examples of heterocycloalkyl include, but are not limited to, 1-(1,2,5,6-tetrahydropyridyl), 1-piperidinyl, 2-piperidinyl, 3-piperidinyl, 4-morpholinyl, 3-morpholinyl, tetrahydrofuran-2-yl, tetrahydrofuran-3-yl, tetrahydrothien-2-yl, tetrahydrothien-3-yl, 1-piperazinyl, 2-piperazinyl, and the like. The terms "cycloalkylene" and "heterocycloalkylene" refer to the divalent derivatives of cycloalkyl and heterocycloalkyl, respectively.

The term "aryl" means, unless otherwise stated, a polyunsaturated, aromatic, hydrocarbon substituent which can be a single ring or multiple rings (preferably from 1 to 3 rings) which are fused together (e.g. naphthyl) or linked covalently. The term "heteroaryl" refers to aryl groups (or rings) that contain heteroatoms (in at least one ring in the case of multiple rings) selected from N, O, and S, wherein the nitrogen

and sulfur atoms are optionally oxidized, and the nitrogen atom(s) are optionally quaternized. A heteroaryl group can be attached to the remainder of the molecule through a carbon or heteroatom. Non-limiting examples of aryl and heteroaryl groups include phenyl, 1-naphthyl, 2-naphthyl, 4-biphenyl, 1-pyrrolyl, 2-pyrrolyl, 3-pyrrolyl, 3-pyrazolyl, 2-imidazolyl, 4-imidazolyl, pyrazinyl, 2-oxazolyl, 4-oxazolyl, 2-phenyl-4-oxazolyl, 5-oxazolyl, 3-isoxazolyl, 4-isoxazolyl, 5-isoxazolyl, 2-thiazolyl, 4-thiazolyl, 5-thiazolyl, 2-furyl, 3-furyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, 2-pyrimidyl, 4-pyrimidyl, 5-benzothiazolyl, purinyl, 2-benzimidazolyl, 5-indolyl, 1-isoquinolyl, 6-isoquinolyl, 2-quinoxalinylnyl, 5-quinoxalinylnyl, 3-quinolyl, and 6-quinolyl. Thus, the term "heteroaryl" include fused ring structures in which at least one ring includes at least two double bonds. Substituents for each of above noted aryl and heteroaryl ring systems are selected from the group of acceptable substituents described below. The terms "arylene" and "heteroarylene" refer to the divalent radicals of aryl and heteroaryl, respectively.

For brevity, the term "aryl" when used in combination with other terms (e.g., aryloxo, arylthioxo, arylalkyl) includes both aryl and heteroaryl rings as defined above. Thus, the term "arylalkyl" is meant to include those radicals in which an aryl group is attached to an alkyl group (e.g., benzyl, phenethyl, pyridylmethyl and the like) including those alkyl groups in which a carbon atom (e.g., a methylene group) has been replaced by, for example, an oxygen atom (e.g., phenoxymethyl, 2-pyridyloxymethyl, 3-(1-naphthyloxy)propyl, and the like). However, the term "haloaryl," as used herein is meant to cover only aryls substituted with one or more halogens.

Where a heteroalkyl, heterocycloalkyl, or heteroaryl includes a specific number of members (e.g., "3 to 7 membered"), the term "member" refers to a carbon or heteroatom.

The term "oxo" as used herein means an oxygen that is double bonded to a carbon atom.

Each of above terms (e.g., "alkyl," "heteroalkyl," "cycloalkyl", and "heterocycloalkyl", "heteroaryl" as well as their divalent radical derivatives) are meant to include both substituted and unsubstituted forms of the indicated radical. Preferred substituents for each type of radical are provided below.

Substituents for alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl monovalent and divalent derivative radicals (including those groups often referred to as alkylene, alkenyl, heteroalkylene, heteroalkenyl, alkynyl, cycloalkyl, heterocycloalkyl, cycloalkenyl, and heterocycloalkenyl) can be one or more of a variety of groups selected from, but not limited to:  $-\text{OR}'$ ,  $=\text{O}$ ,  $=\text{NR}'$ ,  $=\text{N}-\text{OR}'$ ,  $-\text{NR}'\text{R}''$ ,  $-\text{SR}'$ ,  $-\text{halogen}$ ,  $-\text{SiR}'\text{R}''\text{R}'''$ ,  $-\text{OC}(\text{O})\text{R}'$ ,  $-\text{C}(\text{O})\text{R}'$ ,  $-\text{CO}_2\text{R}'$ ,  $-\text{C}(\text{O})\text{NR}'\text{R}''$ ,  $-\text{OC}(\text{O})\text{NR}'\text{R}''$ ,  $-\text{NR}''\text{C}(\text{O})\text{R}'$ ,  $-\text{NR}'-\text{C}(\text{O})\text{NR}''\text{R}'''$ ,  $-\text{NR}''\text{C}(\text{O})\text{OR}'$ ,  $-\text{NR}-\text{C}(\text{NR}'\text{R}''')=\text{NR}'''$ ,  $-\text{S}(\text{O})\text{R}'$ ,  $-\text{S}(\text{O})_2\text{R}'$ ,  $-\text{S}(\text{O})_2\text{NR}'\text{R}''$ ,  $-\text{NRSO}_2\text{R}'$ ,  $-\text{CN}$  and  $-\text{NO}_2$  in a number ranging from zero to  $(2m'+1)$ , where  $m'$  is the total number of carbon atoms in such radical.  $\text{R}'$ ,  $\text{R}''$ ,  $\text{R}'''$  and  $\text{R}''''$  each preferably independently refer to hydrogen, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl (e.g., aryl substituted with 1-3 halogens), substituted or unsubstituted alkyl, alkoxy or thioalkoxy groups, or arylalkyl groups. As used herein, an "alkoxy" group is an alkyl attached to the remainder of the molecule through a divalent oxygen radical. When a compound of the invention includes more than one R group, for example, each of the R groups is independently selected as are each  $\text{R}'$ ,  $\text{R}''$ ,  $\text{R}'''$  and  $\text{R}''''$  groups when more than one of these groups is present. When  $\text{R}'$  and  $\text{R}''$  are attached to the

same nitrogen atom, they can be combined with the nitrogen atom to form a 4-, 5-, 6-, or 7-membered ring. For example,  $-\text{NR}'\text{R}''$  is meant to include, but not be limited to, 1-pyrrolidinyl and 4-morpholinyl. From the above discussion of substituents, one of skill in the art will understand that the term "alkyl" is meant to include groups including carbon atoms bound to groups other than hydrogen groups, such as haloalkyl (e.g.,  $-\text{CF}_3$  and  $-\text{CH}_2\text{CF}_3$ ) and acyl (e.g.,  $-\text{C}(\text{O})\text{CH}_3$ ,  $-\text{C}(\text{O})\text{CF}_3$ ,  $-\text{C}(\text{O})\text{CH}_2\text{OCH}_3$ , and the like).

Similar to the substituents described for alkyl radicals above, exemplary substituents for aryl and heteroaryl groups (as well as their divalent derivatives) are varied and are selected from, for example: halogen,  $-\text{OR}'$ ,  $-\text{NR}'\text{R}''$ ,  $-\text{SR}'$ ,  $-\text{halogen}$ ,  $-\text{SiR}'\text{R}''\text{R}'''$ ,  $-\text{OC}(\text{O})\text{R}'$ ,  $-\text{C}(\text{O})\text{R}'$ ,  $-\text{CO}_2\text{R}'$ ,  $-\text{C}(\text{O})\text{NR}'\text{R}''$ ,  $-\text{OC}(\text{O})\text{NR}'\text{R}''$ ,  $-\text{NR}''\text{C}(\text{O})\text{R}'$ ,  $-\text{NR}'-\text{C}(\text{O})\text{NR}''\text{R}'''$ ,  $-\text{NR}''\text{C}(\text{O})\text{OR}'$ ,  $-\text{NR}-\text{C}(\text{NR}'\text{R}''')=\text{NR}'''$ ,  $-\text{NR}-\text{C}(\text{NR}'\text{R}''')=\text{NR}'''$ ,  $-\text{S}(\text{O})\text{R}'$ ,  $-\text{S}(\text{O})_2\text{R}'$ ,  $-\text{S}(\text{O})_2\text{NR}'\text{R}''$ ,  $-\text{NRSO}_2\text{R}'$ ,  $-\text{CN}$  and  $-\text{NO}_2$ ,  $-\text{R}'$ ,  $-\text{CH}(\text{Ph})_2$ , fluoro( $\text{C}_1$ - $\text{C}_4$ )alkoxy, and fluoro( $\text{C}_1$ - $\text{C}_4$ )alkyl, in a number ranging from zero to the total number of open valences on aromatic ring system; and where  $\text{R}'$ ,  $\text{R}''$ ,  $\text{R}'''$  and  $\text{R}''''$  are preferably independently selected from hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl and substituted or unsubstituted heteroaryl. When a compound of the invention includes more than one R group, for example, each of the R groups is independently selected as are each  $\text{R}'$ ,  $\text{R}''$ ,  $\text{R}'''$  and  $\text{R}''''$  groups when more than one of these groups is present.

Two of the substituents on adjacent atoms of the aryl or heteroaryl ring may optionally form a ring of the formula  $-\text{T}-\text{C}(\text{O})-(\text{CRR}')_q-\text{U}-$ , wherein T and U are independently  $-\text{NR}-$ ,  $-\text{O}-$ ,  $-\text{CRR}'$  or a single bond, and q is an integer of from 0 to 3. Alternatively, two of the substituents on adjacent atoms of the aryl or heteroaryl ring may optionally be replaced with a substituent of the formula  $-\text{A}-(\text{CH}_2)_r-\text{B}-$ , wherein A and B are independently  $-\text{CRR}'$ ,  $-\text{O}-$ ,  $-\text{NR}-$ ,  $-\text{S}-$ ,  $-\text{S}(\text{O})-$ ,  $-\text{S}(\text{O})_2-$ ,  $-\text{S}(\text{O})_2\text{NR}'$  or a single bond, and r is an integer of from 1 to 4. One of the single bonds of the new ring so formed may optionally be replaced with a double bond. Alternatively, two of the substituents on adjacent atoms of the aryl or heteroaryl ring may optionally be replaced with a substituent of the formula  $-(\text{CRR}')_s-\text{X}'-(\text{C}''\text{R}''')_d-$ , where s and d are independently integers of from 0 to 3, and  $\text{X}'$  is  $-\text{O}-$ ,  $-\text{NR}'$ ,  $-\text{S}-$ ,  $-\text{S}(\text{O})-$ ,  $-\text{S}(\text{O})_2-$ , or  $-\text{S}(\text{O})_2\text{NR}'$ . The substituents  $\text{R}$ ,  $\text{R}'$ ,  $\text{R}''$  and  $\text{R}'''$  are preferably independently selected from hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, and substituted or unsubstituted heteroaryl.

As used herein, the term "heteroatom" or "ring heteroatom" is meant to include oxygen (O), nitrogen (N), sulfur (S), phosphorus (P), and silicon (Si).

The terms "halo" or "halogen," by themselves or as part of another substituent, mean, unless otherwise stated, a fluorine, chlorine, bromine, or iodine atom. Additionally, terms such as "haloalkyl," are meant to include monohaloalkyl and polyhaloalkyl. For example, the term "halo( $\text{C}_1$ - $\text{C}_4$ )alkyl" is meant to include, but not be limited to, trifluoromethyl, 2,2,2-trifluoroethyl, 4-chlorobutyl, 3-bromopropyl, and the like.

A "size-limited substituent" or "size-limited substituent group," as used herein means a group selected from all of the substituents described above for a "substituent group," wherein each substituted or unsubstituted alkyl is a substituted or unsubstituted  $\text{C}_1$ - $\text{C}_{20}$  each substituted or unsubstituted

tuted heteroalkyl is a substituted or unsubstituted 2 to 20 membered heteroalkyl, each substituted or unsubstituted cycloalkyl is a substituted or unsubstituted C<sub>4</sub>-C<sub>8</sub> cycloalkyl, and each substituted or unsubstituted heterocycloalkyl is a substituted or unsubstituted 4 to 8 membered heterocycloalkyl.

A “lower substituent” or “lower substituent group,” as used herein means a group selected from all of the substituents described above for a “substituent group,” wherein each substituted or unsubstituted alkyl is a substituted or unsubstituted C<sub>1</sub>-C<sub>8</sub> alkyl, each substituted or unsubstituted heteroalkyl is a substituted or unsubstituted 2 to 8 membered heteroalkyl, each substituted or unsubstituted cycloalkyl is a substituted or unsubstituted C<sub>5</sub>-C<sub>7</sub> cycloalkyl, and each substituted or unsubstituted heterocycloalkyl is a substituted or unsubstituted 5 to 7 membered heterocycloalkyl.

In some embodiments, each substituted group described in the compounds herein is substituted with at least one substituent group. More specifically, in some embodiments, each substituted alkyl, substituted heteroalkyl, substituted cycloalkyl, substituted heterocycloalkyl, substituted aryl, substituted heteroaryl, substituted alkylene, substituted heteroalkylene, substituted cycloalkylene, substituted heterocycloalkylene, substituted arylene, and/or substituted heteroarylene described in the compounds herein are substituted with at least one substituent group. In other embodiments, at least one or all of these groups are substituted with at least one size-limited substituent group. Alternatively, at least one or all of these groups are substituted with at least one lower substituent group.

In other embodiments of the compounds herein, each substituted or unsubstituted alkyl is a substituted or unsubstituted C<sub>1</sub>-C<sub>20</sub> alkyl, each substituted or unsubstituted heteroalkyl is a substituted or unsubstituted 2 to 20 membered heteroalkyl, each substituted or unsubstituted cycloalkyl is a substituted or unsubstituted C<sub>3</sub>-C<sub>8</sub> cycloalkyl, and/or each substituted or unsubstituted heterocycloalkyl is a substituted or unsubstituted 3 to 8 membered heterocycloalkyl. In some embodiments of the compounds herein, each substituted or unsubstituted alkylene is a substituted or unsubstituted C<sub>1</sub>-C<sub>20</sub> alkylene, each substituted or unsubstituted heteroalkylene is a substituted or unsubstituted 2 to 20 membered heteroalkylene, each substituted or unsubstituted cycloalkylene is a substituted or unsubstituted C<sub>3</sub>-C<sub>8</sub> cycloalkylene, and/or each substituted or unsubstituted heterocycloalkylene is a substituted or unsubstituted 3 to 8 membered heterocycloalkylene.

In some embodiments, each substituted or unsubstituted alkyl is a substituted or unsubstituted C<sub>1</sub>-C<sub>8</sub> alkyl, each substituted or unsubstituted heteroalkyl is a substituted or unsubstituted 2 to 8 membered heteroalkyl, each substituted or unsubstituted cycloalkyl is a substituted or unsubstituted C<sub>5</sub>-C<sub>7</sub> cycloalkyl, and/or each substituted or unsubstituted heterocycloalkyl is a substituted or unsubstituted 5 to 7 membered heterocycloalkyl. In some embodiments, each substituted or unsubstituted alkylene is a substituted or unsubstituted C<sub>1</sub>-C<sub>8</sub> alkylene, each substituted or unsubstituted heteroalkylene is a substituted or unsubstituted 2 to 8 membered heteroalkylene, each substituted or unsubstituted cycloalkylene is a substituted or unsubstituted C<sub>5</sub>-C<sub>7</sub> cycloalkylene, and/or each substituted or unsubstituted heterocycloalkylene is a substituted or unsubstituted 5 to 7 membered heterocycloalkylene. In some embodiments, the compound is a chemical species set forth in the Examples section below.

A “bulky residue” in an amino acid residue having a side chain group that is larger (i.e. having more atoms and tending to fill more space) than glycine and alanine, and optionally

larger than cysteine. A bulky residue may be methionine, leucine, phenylalanine and threonine. In some embodiments, the bulky residue may be larger than leucine, isoleucine and threonine. In some embodiments, the bulky residue includes a cyclic moiety.

“Electrophilic” is used herein in accordance with its plain ordinary meaning and refers to a chemical group having a tendency to attract, acquire or accept electrons or react at electron-rich sites.

“Nucleophilic” is used herein in accordance with its plain ordinary meaning and refers to a chemical group having a tendency to donate electrons (e.g. lower electron density) or react at electron poor sites.

“Electrophilic moiety” as used herein refers to a functional group or chemical substituent that is electrophilic. Example electrophilic moieties include, but are not limited to vinylsulfonamides, acrylamides, epoxides, and fluoromethylketones.

As defined herein, the term “electrophilic substituent” is a substituent that is electrophilic. An electrophilic substituent, electrophilic moieties and electrophilic chemical groups are typically electron-poor functional groups and can react with an electron-donating group, such as a nucleophile, by accepting an electron pair. In some embodiments, the electrophilic substituent, moiety or chemical group of a compound is capable of reacting with a cysteine residue. In some embodiments, the electrophilic substituent, moiety or chemical group is capable of forming a covalent bond with a cysteine residue within the ATP binding site of the kinase. The covalent bond is usually formed between the electrophilic substituent, moiety or chemical group and the sulfhydryl group of the cysteine and may be a reversible or irreversible bond. In some embodiments, the covalent bond is irreversible.

As used herein, the terms “protein kinase” or “kinase” are used in accordance with its plain ordinary meaning and refer to an enzyme that is capable of phosphorylating an amino acid residue, e.g. an amino acid residue on a protein. Typically specific serine, threonine, or tyrosine residues are phosphorylated. Thus, protein kinase encompasses serine protein kinases, threonine protein kinases, and tyrosine protein kinases. An “inhibitor of a protein kinase” is a compound or agent that reduces the activity of a protein kinase. In some embodiments, a “protein kinase inhibitor” is a compound that reduces the activity of the protein kinase by binding to the protein kinase. Thus, a “protein kinase inhibitor” can inhibit activity of the enzyme in a competitive, or a noncompetitive manner.

As defined herein, the term “cysteine substituted kinase” refers to a recombinant kinase where a gatekeeper amino acid residue (e.g. within an ATP binding site of the kinase) is replaced with a cysteine residue. Similarly, a “glycine substituted kinase” refers to a recombinant kinase where a gatekeeper amino acid residue (e.g. within an ATP binding site of the kinase) is replaced with a glycine residue, and a “alanine substituted kinase” refers to a kinase where a gatekeeper amino acid residue (e.g. within an ATP binding site of the kinase) is replaced with an alanine residue.

As defined herein, the term “fused rings” refers to a ring system with two or more rings having at least one bond and two atoms in common.

The terms “nucleic acid,” “oligonucleotide,” “polynucleotide,” and like terms typically refer to polymers of deoxyribonucleotides or ribonucleotides in either single—or double-stranded form, and complements thereof. The term “nucleotide” typically refers to a monomer. The terms encompass nucleic acids containing known nucleotide analogs or modified backbone residues or linkages, which are synthetic, naturally occurring, and non-naturally occurring,

which have similar binding properties as the reference nucleic acid, and which are metabolized in a manner similar to the reference nucleotides. Examples of such analogs include, without limitation, phosphorothioates, phosphoramidates, methyl phosphonates, chiral-methyl phosphonates, 2-O-methyl ribonucleotides, and peptide-nucleic acids (PNAs).

Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (e.g., degenerate codon substitutions) and complementary sequences, as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer et al., *Nucleic Acid Res.* 19:5081 (1991); Ohtsuka et al., *J. Biol. Chem.* 260:2605-2608 (1985); Rossolini et al., *Mol. Cell. Probes* 8:91-98 (1994)). The term nucleic acid is used interchangeably with gene, cDNA, mRNA, oligonucleotide, and polynucleotide.

Nucleic acids "hybridize" when they associate, typically in solution. Nucleic acids hybridize due to a variety of well-characterized physico-chemical forces, such as hydrogen bonding, solvent exclusion, base stacking and the like. As used herein, the term "stringent hybridization wash conditions" in the context of nucleic acid hybridization experiments, such as Southern and Northern hybridizations, are sequence dependent, and are different under different environmental parameters. An extensive guide to the hybridization of nucleic acids is found in Tijssen, 1993, "Laboratory Techniques in Biochemistry and Molecular Biology-Hybridization with Nucleic Acid Probes," Part I, Chapter 2 (Elsevier, N.Y.), which is incorporated herein by reference.

The terms "peptide," "polypeptide," and "protein" are used interchangeably herein to refer to a polymer of amino acid residues.

The term "amino acid" refers to naturally occurring and synthetic amino acids, as well as amino acid analogs. Naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, e.g., hydroxyproline,  $\gamma$ -carboxyglutamate, and O-phosphoserine. Amino acid analogs refers to compounds that have the same basic chemical structure as a naturally occurring amino acid, i.e., an  $\alpha$ -carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, e.g., homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs have modified R groups (e.g., norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid.

Amino acids may be referred to herein by either their commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Nucleotides, likewise, may be referred to by their commonly accepted single-letter codes.

An amino acid or nucleotide base "position" is denoted by a number that sequentially identifies each amino acid (or nucleotide base) in the reference sequence based on its position relative to the N-terminus (or 5'-end). Due to deletions, insertions, truncations, fusions, and the like that must be taken into account when determining an optimal alignment, in general the amino acid residue number in a test sequence determined by simply counting from the N-terminus will not necessarily be the same as the number of its corresponding position in the reference sequence. For example, in a case where a variant has a deletion relative to an aligned reference sequence, there will be no amino acid in the variant that corresponds to a position in the reference sequence at the site of deletion. Where there is an insertion in an aligned reference

sequence, that insertion will not correspond to a numbered amino acid position in the reference sequence. In the case of truncations or fusions there can be stretches of amino acids in either the reference or aligned sequence that do not correspond to any amino acid in the corresponding sequence.

The terms "numbered with reference to" or "corresponding to," when used in the context of the numbering of a given amino acid or polynucleotide sequence, refers to the numbering of the residues of a specified reference sequence when the given amino acid or polynucleotide sequence is compared to the reference sequence.

A "conservative substitution" as used with respect to amino acids, refers to the substitution of an amino acid with a chemically similar amino acid. Amino acid substitutions which often preserve the structural and/or functional properties of the polypeptide in which the substitution is made are known in the art and are described, for example, by H. Neurath and R. L. Hill, 1979, in "The Proteins," Academic Press, New York. The most commonly occurring exchanges are isoleucine/valine, tyrosine/phenylalanine, aspartic acid/glutamic acid, lysine/arginine, methionine/leucine, aspartic acid/asparagine, glutamic acid/glutamine, leucine/isoleucine, methionine/isoleucine, threonine/serine, tryptophan/phenylalanine, tyrosine/histidine, tyrosine/tryptophan, glutamine/arginine, histidine/asparagine, histidine/glutamine, lysine/asparagine, lysine/glutamine, lysine/glutamic acid, phenylalanine/leucine, phenylalanine/methionine, serine/alanine, serine/asparagine, valine/leucine, and valine/methionine. In some embodiments, there may be at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, or at least 40 conservative substitutions.

The term "amino acid substitution set" or "substitution set" refers to a group of amino acid substitutions. A substitution set can have 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or more amino acid substitutions.

The term "isolated" refers to a nucleic acid, polynucleotide, polypeptide, protein, or other component that is partially or completely separated from components with which it is normally associated (other proteins, nucleic acids, cells, etc.). In some embodiments, an isolated polypeptide or protein is a recombinant polypeptide or protein.

A nucleic acid (such as a polynucleotide), a polypeptide, or a cell is "recombinant" when it is artificial or engineered, or derived from or contains an artificial or engineered protein or nucleic acid (e.g. non-natural or not wild type). For example, a polynucleotide that is inserted into a vector or any other heterologous location, e.g., in a genome of a recombinant organism, such that it is not associated with nucleotide sequences that normally flank the polynucleotide as it is found in nature is a recombinant polynucleotide. A protein expressed in vitro or in vivo from a recombinant polynucleotide is an example of a recombinant polypeptide. Likewise, a polynucleotide sequence that does not appear in nature, for example a variant of a naturally occurring gene, is recombinant.

"Identity" or "percent identity," in the context of two or more polypeptide sequences, refers to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues that are the same (e.g., share at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 88% identity, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity) over a specified region to

a reference sequence, when compared and aligned for maximum correspondence over a comparison window, or designated region as measured using a sequence comparison algorithms or by manual alignment and visual inspection.

Optimal alignment of sequences for comparison and determination of sequence identity can be determined by a sequence comparison algorithm or by visual inspection (see, generally, Ausubel et al., *infra*). When optimally aligning sequences and determining sequence identity by visual inspection, percent sequence identity is calculated as the number of residues of the test sequence that are identical to the reference sequence divided by the number of non-gap positions and multiplied by 100. When using a sequence comparison algorithm, test and reference sequences are entered into a computer, subsequence coordinates and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identities for the test sequences relative to the reference sequence, based on the program parameters as known in the art, for example BLAST or BLAST 2.0. For example, comparison can be conducted, e.g., by the local homology algorithm of Smith & Waterman, 1981, *Adv. Appl. Math.* 2:482, by the homology alignment algorithm of Needleman & Wunsch, 1970, *J. Mol. Biol.* 48:443, by the search for similarity method of Pearson & Lipman, 1988, *Proc. Nat'l. Acad. Sci. USA* 85:2444, or by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, Wis.). Thus alignment can be carried out for sequences that have deletions and/or additions, as well as those that have substitutions, as well as naturally occurring, e.g., polymorphic or allelic variants, and man-made variants.

The phrase "substantial sequence identity" or "substantial identity," in the context of two nucleic acid or polypeptide sequences, refers to a sequence that has at least 70% identity to a reference sequence. Percent identity can be any integer from 70% to 100%. Two nucleic acid or polypeptide sequences that have 100% sequence identity are said to be "identical." A nucleic acid or polypeptide sequence are said to have "substantial sequence identity" to a reference sequence when the sequences have at least about 70%, at least about 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% or greater sequence identity as determined using the methods described herein, such as BLAST using standard parameters as described above.

The term "pre-protein" refers to a protein including an amino-terminal signal peptide (or leader sequence) region attached. The signal peptide is cleaved from the pre-protein by a signal peptidase prior to secretion to result in the "mature" or "secreted" protein.

A "vector" is a DNA construct for introducing a DNA sequence into a cell. A vector may be an expression vector

that is operably linked to a suitable control sequence capable of effecting the expression in a suitable host of the polypeptide encoded in the DNA sequence. An "expression vector" has a promoter sequence operably linked to the DNA sequence (e.g., transgene) to drive expression in a host cell, and in some embodiments a transcription terminator sequence.

The term "expression" includes any step involved in the production of the polypeptide including, but not limited to, transcription, post-transcriptional modification, translation, post-translational modification, and secretion.

The term "operably linked" refers to a configuration in which a control sequence is appropriately placed at a position relative to the coding sequence of the DNA sequence such that the control sequence influences the expression of a polypeptide.

An amino acid or nucleotide sequence (e.g., a promoter sequence, signal peptide, terminator sequence, etc.) is "heterologous" to another sequence with which it is operably linked if the two sequences are not associated in nature.

The terms "transform" or "transformation," as used in reference to a cell, means a cell has a non-native nucleic acid sequence integrated into its genome or as an episome (e.g., plasmid) that is maintained through multiple generations.

The term "culturing" refers to growing a population of microbial cells under suitable conditions in a liquid or solid medium.

The term "introduced," as used in the context of inserting a nucleic acid sequence into a cell, means conjugated, transfected, transduced or transformed (collectively "transformed") or otherwise incorporated into the genome of, or maintained as an episome in, the cell.

As defined herein, the term "gatekeeper amino acid residue" or "gatekeeper residue" refers to a residue (e.g. within the ATP binding site of a kinase) that is capable of controlling or modulating the ability of a kinase substrate to bind to the kinase. For example, in some embodiments, the accessibility of a protein kinase substrate to the ATP binding site is controlled by the gatekeeper residue. In certain embodiments, the gatekeeper residue controls the ability of the substrate to access or bind a hydrophobic pocket adjacent to the ATP binding site. (Elphick et al. *ACS Chemical Biology*, 2:299-314, 2007). As defined herein, a natural gatekeeper residue refers to a gatekeeper residue identified in a wild-type kinase. Examples of gatekeeper residues include, e.g., Thr338 of c-Src (v-Src numbering, see Liu et al., *Chemistry & Biology*, 6:671-678, 1999), and Thr 493 of rsk2 (see US Application No. 2009/0221614). Gatekeeper residues in other kinases, e.g., gatekeeper residues corresponding to Thr338 of c-Src can be readily identified by structure-based sequence alignment of kinase domain of various src or non-src kinases. The following is a structure-based sequence alignment of several kinase domains (see U.S. Patent Publication No. 2009/0221614). The gatekeeper residues referred to herein are highlighted in bold italics:

Name	Sequence	SEQ ID NO:
src	---PEAFLQEAQVMK--KLRHEKLVQLYAVVSEEP---IYIV <b>TE</b> YM	52
rsk2	---KRDPTTEEIEILLR-YGQHPNIITLKDVEDDGKY--VYVV <b>TE</b> LM	53
nek2	-EVEKQMLVSEVNLLR--ELKHPNIVRYDRIIDRTNTTLYIV <b>ME</b> YC	54
mekk1	QEEVVEALRREEIRMMS--HLNHPNIIRMLGATCEKSN--YNL <b>FE</b> WM	55

Name	Sequence	SEQ ID NO :
msk1	--MEANTQKEITALK-LCEGHPNIVKLHEVFHDQLH--TFLV <b><u>M</u></b> ELL	56
plk1	-PHQREKMSMEISIHR--SLAHQHVVGPHGFFEDNDF--VFVV <b><u>L</u></b> ELC	57

Additional gatekeeper residues in various kinases can be identified by sequence alignment (see Liu et al., Chemistry & Biology, 6:671-678, 1999). For example, gatekeeper residues

in various kinases corresponding to Thr338 of v-Src are highlighted in bold underlined in the sequence alignment below:

		338 ↓ Sequence 338 ↓		SEQ ID NO :
Name	Start			
v-Src	(318)	RHEKLVQLYAMVSE-----EPIYIV <b><u>T</u></b> EYMSK--GSLLDFLKGEMGKY		58
c-Src	(318)	RHEKLVQLYAVVSE-----EPIYIV <b><u>T</u></b> EYMSK--GSLLDFLKGETGKY		59
Lck	(296)	QHQLVRLRYAVVTQ-----EPIYI <b><u>I</u></b> EYMEN--GSLVDFLKTPSGIK		60
Fyn	(319)	KHDKLVQLYAVVSE-----EPIYIV <b><u>T</u></b> EYMNK--GSLLDFLKDGEGRA		61
c-Yes	(325)	RHDKLVPLYAVVSE-----EPIYIV <b><u>T</u></b> EFMSK--GSLLDFLKEGDGKY		62
Yrk	(318)	RHDKLVQLYAVVSE-----EPIYIV <b><u>T</u></b> EFMSQ--GSLLDFLKDGDGRY		63
c-Fgr	(311)	RHDKLVQLYAVVSE-----EPIYIV <b><u>T</u></b> EFMCH--GSLLDFLKNPEGQD		64
Lyn	(295)	QHDKLVRLYAVVTRE-----EPIYI <b><u>I</u></b> EYMAK--GSLLDFLKSDEGGK		65
Hck	(318)	QHDKLVKLHAVVTK-----EPIYI <b><u>I</u></b> EFMAK--GSLLDFLKSDEGSK		66
Blk	(287)	QHERLVRLYAVVTR-----EPIYIV <b><u>T</u></b> EYMAR--GCLLDFLKTDEGSR		67
Ab1	(313)	KHPNLVQLLGVCVCTRE-----PPFYI <b><u>I</u></b> EFMTY--GNLLDYLRECNRQE		68
Btk	(473)	SHEKLVQLYGVCTKQ-----RPIFI <b><u>I</u></b> EYMAN--GCLLNLYLRMRHR		69
Csk	(244)	RHSNLVQLLGIVVEEK-----GGLYIV <b><u>T</u></b> EYMAK--GSLVDYLRSRGRSV		70
PDGFR	(660)	PHLNVVNLLGACTKG-----GPIYI <b><u>I</u></b> EYCRY--GDLVDYLHRNKHTF		71
p38	(85)	GLLDVFTPARSLLEEF-----NDVVLV <b><u>T</u></b> HLMGA---DLNNIVKCQKLTDD		72
ZAP-70	(394)	DNPYIVRLIGVCQA-----EALMLV <b><u>V</u></b> EMAGG--GPLHKFL-VGKREE		73
JAK2	(906)	QHDNIVKYKGVCSAGR-----RNLRLI <b><u>M</u></b> EYLPY--GSLRDYLQKHKER		74
PKA	(99)	NFPFLVKLEFSFKDN-----SNLYMV <b><u>V</u></b> EYVPG--GEMFSHLRRIGR		75
CamK II	(68)	KHPNIVRLHDSISEE-----GHHYLI <b><u>R</u></b> DLVTG--GELFEDIVAREY		76
Cdk2	(59)	NHPNIVKLLDVIHTE-----NKLVLV <b><u>T</u></b> EFLHQ---DLKKFMDASALTG		77

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"Control" or "control experiment" is used in accordance with its plain ordinary meaning and refers to an experiment in which the subjects or reagents of the experiment are treated as in a parallel experiment except for omission of a procedure, reagent, or variable of the experiment. In some instances, the control is used as a standard of comparison in evaluating experimental effects.

"Contacting" is used in accordance with its plain ordinary meaning and refers to the process of allowing at least two distinct species (e.g. chemical compounds including biomolecules, or cells) to become sufficiently proximal to react, interact or physically touch. It should be appreciated, however, the resulting reaction product can be produced directly from a reaction between the added reagents or from an intermediate from one or more of the added reagents which can be produced in the reaction mixture. The term "contacting" includes incubating an inhibitor with the kinase.

As defined herein, the term "inhibition", "inhibit", "inhibiting" and the like in reference to a kinase-inhibitor interaction means negatively affecting (e.g. decreasing) the activity of the kinase relative to the activity of the kinase in the absence of the inhibitor. Thus, inhibition includes, at least in part, partially or totally blocking stimulation, decreasing, preventing, or delaying activation, or inactivating, desensitizing, or down-regulating signal transduction. Similarly an "inhibitor" is a compound that inhibits kinase activity, e.g., by binding, partially or totally block stimulation, decrease, prevent, or delay activation, or inactivate, desensitize, or down-regulate signal transduction.

"Disease" or "condition" refer a state of being or health status of a patient or subject capable of being treated with the compounds provided herein. Examples of disorders or conditions include, but are not limited to, cancer, cardiovascular disease, hypertension, Syndrome X, depression, anxiety, glaucoma, human immunodeficiency virus (HIV) or acquired immunodeficiency syndrome (AIDS), neurodegeneration, Alzheimer's disease, Parkinson's disease, cognition enhancement, Cushing's Syndrome, Addison's Disease, osteoporosis, frailty, muscle frailty, inflammatory diseases, osteoarthritis, rheumatoid arthritis, asthma and rhinitis, adrenal function-related ailments, viral infection, immunodeficiency, immunomodulation, autoimmune diseases, allergies, wound healing, compulsive behavior, multi-drug resistance, addiction, psychosis, anorexia, cachexia, post-traumatic stress syndrome, post-surgical bone fracture, medical catabolism, major psychotic depression, mild cognitive impairment, psychosis, dementia, hyperglycemia, stress disorders, antipsychotic induced weight gain, delirium, cognitive impairment in depressed patients, cognitive deterioration in individuals with Down's syndrome, psychosis associated with interferon-alpha therapy, chronic pain, pain associated with gastroesophageal reflux disease, postpartum psychosis, postpartum depression, neurological disorders in premature infants, and migraine headaches. In some instances, "disease" or "condition" refer to cancer. In some further instances, "cancer" refers to human cancers and carcinomas, sarcomas, adenocarcinomas, lymphomas, leukemias, etc., including solid and lymphoid cancers, kidney, breast, lung, bladder, colon, ovarian, prostate, pancreas, stomach, brain, head and neck, skin, uterine, testicular, glioma, esophagus, and liver cancer, including hepatocarcinoma, lymphoma, including B-acute lymphoblastic lymphoma, non-Hodgkin's lymphomas (e.g., Burkitt's, Small Cell, and Large Cell lymphomas) and Hodgkin's lymphoma, leukemia (including AML, ALL, and CML), and multiple myeloma.

"Patient" or "subject in need thereof" refers to a living organism suffering from or prone to a condition that can be

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treated by administration of a pharmaceutical composition as provided herein. Non-limiting examples include humans, other mammals and other non-mammalian animals.

Abbreviations used herein have their conventional meaning within the chemical and biological arts.

Where substituent groups are specified by their conventional chemical formulae, written from left to right, they equally encompass the chemically identical substituents that would result from writing the structure from right to left, e.g.,  $-\text{CH}_2\text{O}-$  is equivalent to  $-\text{OCH}_2-$ .

As used herein, the symbol,



indicates the point of attachment of a substituents to the remainder of a molecule.

A "substituent group," as used herein, means a group selected from the following moieties:

(A)  $-\text{OH}$ ,  $-\text{NH}_2$ ,  $-\text{SH}$ ,  $-\text{CN}$ ,  $-\text{CF}_3$ ,  $-\text{NO}_2$ , oxo, halogen, unsubstituted alkyl, unsubstituted heteroalkyl, unsubstituted cycloalkyl, unsubstituted heterocycloalkyl, unsubstituted aryl, unsubstituted heteroaryl, and

(B) alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, and heteroaryl, substituted with at least one substituent selected from:

(i) oxo,  $-\text{OH}$ ,  $-\text{NH}_2$ ,  $-\text{SH}$ ,  $-\text{CN}$ ,  $-\text{CF}_3$ ,  $-\text{NO}_2$ , halogen, unsubstituted alkyl, unsubstituted heteroalkyl, unsubstituted cycloalkyl, unsubstituted heterocycloalkyl, unsubstituted aryl, unsubstituted heteroaryl, and

(ii) alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, and heteroaryl, substituted with at least one substituent selected from:

(a) oxo,  $-\text{OH}$ ,  $-\text{NH}_2$ ,  $-\text{SH}$ ,  $-\text{CN}$ ,  $-\text{CF}_3$ ,  $-\text{NO}_2$ , halogen, unsubstituted alkyl, unsubstituted heteroalkyl, unsubstituted cycloalkyl, unsubstituted heterocycloalkyl, unsubstituted aryl, unsubstituted heteroaryl, and

(b) alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, or heteroaryl, substituted with at least one substituent selected from oxo,  $-\text{OH}$ ,  $-\text{NH}_2$ ,  $-\text{SH}$ ,  $-\text{CN}$ ,  $-\text{CF}_3$ ,  $-\text{NO}_2$ , halogen, unsubstituted alkyl, unsubstituted heteroalkyl, unsubstituted cycloalkyl, unsubstituted heterocycloalkyl, unsubstituted aryl, and unsubstituted heteroaryl.

A "size-limited substituent" or "size-limited substituent group," as used herein means a group selected from all of the substituents described above for a "substituent group," wherein each substituted or unsubstituted alkyl is a substituted or unsubstituted  $\text{C}_1$ - $\text{C}_{20}$  alkyl, each substituted or unsubstituted heteroalkyl is a substituted or unsubstituted 2 to 20 membered heteroalkyl, each substituted or unsubstituted cycloalkyl is a substituted or unsubstituted  $\text{C}_4$ - $\text{C}_8$  cycloalkyl, and each substituted or unsubstituted heterocycloalkyl is a substituted or unsubstituted 4 to 8 membered heterocycloalkyl.

A "lower substituent" or "lower substituent group," as used herein means a group selected from all of the substituents described above for a "substituent group," wherein each substituted or unsubstituted alkyl is a substituted or unsubstituted  $\text{C}_1$ - $\text{C}_8$  alkyl, each substituted or unsubstituted heteroalkyl is a substituted or unsubstituted 2 to 8 membered heteroalkyl, each substituted or unsubstituted cycloalkyl is a substituted or unsubstituted  $\text{C}_5$ - $\text{C}_7$  cycloalkyl, and each substituted or unsubstituted heterocycloalkyl is a substituted or unsubstituted 5 to 7 membered heterocycloalkyl.

The compounds of the present invention may exist as salts. The present invention includes such salts. Examples of applicable salt forms include hydrochlorides, hydrobromides, sulfates, methanesulfonates, nitrates, maleates, acetates, citrates, fumarates, tartrates (eg (+)-tartrates, (-)-tartrates or mixtures thereof including racemic mixtures, succinates, benzoates and salts with amino acids such as glutamic acid. These salts may be prepared by methods known to those skilled in art. Also included are base addition salts such as sodium, potassium, calcium, ammonium, organic amino, or magnesium salt, or a similar salt. When compounds of the present invention contain relatively basic functionalities, acid addition salts can be obtained by contacting the neutral form of such compounds with a sufficient amount of the desired acid, either neat or in a suitable inert solvent. Examples of acceptable acid addition salts include those derived from inorganic acids like hydrochloric, hydrobromic, nitric, carbonic, monohydrogencarbonic, phosphoric, monohydrogenphosphoric, dihydrogenphosphoric, sulfuric, monohydrogensulfuric, hydriodic, or phosphorous acids and the like, as well as the salts derived from organic acids like acetic, propionic, isobutyric, maleic, malonic, benzoic, succinic, suberic, fumaric, lactic, mandelic, phthalic, benzenesulfonic, p-tolylsulfonic, citric, tartaric, methanesulfonic, and the like. Also included are salts of amino acids such as arginate and the like, and salts of organic acids like glucuronic or galactunoric acids and the like. Certain specific compounds of the present invention contain both basic and acidic functionalities that allow the compounds to be converted into either base or acid addition salts.

The neutral forms of the compounds are preferably regenerated by contacting the salt with a base or acid and isolating the parent compound in the conventional manner. The parent form of the compound differs from the various salt forms in certain physical properties, such as solubility in polar solvents.

Certain compounds of the present invention can exist in unsolvated forms as well as solvated forms, including hydrated forms. In general, the solvated forms are equivalent to unsolvated forms and are encompassed within the scope of the present invention. Certain compounds of the present invention may exist in multiple crystalline or amorphous forms. In general, all physical forms are equivalent for the uses contemplated by the present invention and are intended to be within the scope of the present invention.

Certain compounds of the present invention possess asymmetric carbon atoms (optical or chiral centers) or double bonds; the enantiomers, racemates, diastereomers, tautomers, geometric isomers, stereoisomeric forms that may be defined, in terms of absolute stereochemistry, as (R)- or (S)-, or as (D)- or (L)- for amino acids, and individual isomers are encompassed within the scope of the present invention. The compounds of the present invention do not include those which are known in art to be too unstable to synthesize and/or isolate. The present invention is meant to include compounds in racemic and optically pure forms. Optically active (R)- and (S)-, or (D)- and (L)-isomers may be prepared using chiral synthons or chiral reagents, or resolved using conventional techniques. When the compounds described herein contain olefinic bonds or other centers of geometric asymmetry, and unless specified otherwise, it is intended that the compounds include both E and Z geometric isomers.

The term "tautomer," as used herein, refers to one of two or more structural isomers which exist in equilibrium and which are readily converted from one isomeric form to another.

It will be apparent to one skilled in the art that certain compounds of this invention may exist in tautomeric forms, all such tautomeric forms of the compounds being within the scope of the invention.

Unless otherwise stated, structures depicted herein are also meant to include all stereochemical forms of the structure; i.e., the R and S configurations for each asymmetric center. Therefore, single stereochemical isomers as well as enantiomeric and diastereomeric mixtures of the present compounds are within the scope of the invention.

Unless otherwise stated, structures depicted herein are also meant to include compounds which differ only in the presence of one or more isotopically enriched atoms. For example, compounds having the present structures except for the replacement of a hydrogen by a deuterium or tritium, or the replacement of a carbon by  $^{13}\text{C}$ - or  $^{14}\text{C}$ -enriched carbon are within the scope of this invention.

The compounds of the present invention may also contain unnatural proportions of atomic isotopes at one or more of atoms that constitute such compounds. For example, the compounds may be radiolabeled with radioactive isotopes, such as for example tritium ( $^3\text{H}$ ), iodine-125 ( $^{125}\text{I}$ ) or carbon-14 ( $^{14}\text{C}$ ). All isotopic variations of the compounds of the present invention, whether radioactive or not, are encompassed within the scope of the present invention.

The term "pharmaceutically acceptable salts" is meant to include salts of active compounds which are prepared with relatively nontoxic acids or bases, depending on the particular substituent moieties found on the compounds described herein. When compounds of the present invention contain relatively acidic functionalities, base addition salts can be obtained by contacting the neutral form of such compounds with a sufficient amount of the desired base, either neat or in a suitable inert solvent. Examples of pharmaceutically acceptable base addition salts include sodium, potassium, calcium, ammonium, organic amino, or magnesium salt, or a similar salt. When compounds of the present invention contain relatively basic functionalities, acid addition salts can be obtained by contacting the neutral form of such compounds with a sufficient amount of the desired acid, either neat or in a suitable inert solvent. Examples of pharmaceutically acceptable acid addition salts include those derived from inorganic acids like hydrochloric, hydrobromic, nitric, carbonic, monohydrogencarbonic, phosphoric, monohydrogenphosphoric, dihydrogenphosphoric, sulfuric, monohydrogensulfuric, hydriodic, or phosphorous acids and the like, as well as the salts derived from relatively nontoxic organic acids like acetic, propionic, isobutyric, maleic, malonic, benzoic, succinic, suberic, fumaric, lactic, mandelic, phthalic, benzenesulfonic, p-tolylsulfonic, citric, tartaric, methanesulfonic, and the like. Also included are salts of amino acids such as arginate and the like, and salts of organic acids like glucuronic or galactunoric acids and the like (see, for example, Berge et al., "Pharmaceutical Salts", *Journal of Pharmaceutical Science*, 1977, 66, 1-19). Certain specific compounds of the present invention contain both basic and acidic functionalities that allow the compounds to be converted into either base or acid addition salts.

In addition to salt forms, the present invention provides compounds, which are in a prodrug form. Prodrugs of the compounds described herein are those compounds that readily undergo chemical changes under physiological conditions to provide the compounds of the present invention. Additionally, prodrugs can be converted to the compounds of the present invention by chemical or biochemical methods in an ex vivo environment. For example, prodrugs can be slowly

converted to the compounds of the present invention when placed in a transdermal patch reservoir with a suitable enzyme or chemical reagent.

The terms “a,” “an,” or “a(n),” when used in reference to a group of substituents herein, mean at least one. For example, where a compound is substituted with “an” alkyl or aryl, the compound is optionally substituted with at least one alkyl and/or at least one aryl. Moreover, where a moiety is substituted with an R substituent, the group may be referred to as “R-substituted.” Where a moiety is R-substituted, the moiety is substituted with at least one R substituent and each R substituent is optionally different.

Description of compounds of the present invention are limited by principles of chemical bonding known to those skilled in the art. Accordingly, where a group may be substituted by one or more of a number of substituents, such substitutions are selected so as to comply with principles of chemical bonding and to give compounds which are not inherently unstable and/or would be known to one of ordinary skill in the art as likely to be unstable under ambient conditions, such as aqueous, neutral, and several known physiological conditions. For example, a heterocycloalkyl or heteroaryl is attached to the remainder of the molecule via a ring heteroatom in compliance with principles of chemical bonding known to those skilled in the art thereby avoiding inherently unstable compounds.

The terms “treating” or “treatment” refers to any indicia of success in the treatment or amelioration of an injury, pathology or condition, including any objective or subjective parameter such as abatement; remission; diminishing of symptoms or making the injury, pathology or condition more tolerable to the patient; slowing in the rate of degeneration or decline; making the final point of degeneration less debilitating; improving a patient’s physical or mental well-being. The treatment or amelioration of symptoms can be based on objective or subjective parameters; including the results of a physical examination, neuropsychiatric exams, and/or a psychiatric evaluation. For example, the certain methods presented herein successfully treat cancer by decreasing the incidence of cancer and/or causing remission of cancer.

An “effective amount” is an amount sufficient to contribute to the treatment, prevention, or reduction of a symptom or symptoms of a disease. An “effective amount” may also be referred to as a “therapeutically effective amount.” A “reduction” of a symptom or symptoms (and grammatical equivalents of this phrase) means decreasing of the severity or frequency of the symptom(s), or elimination of the symptom(s). A “prophylactically effective amount” of a drug is an amount of a drug that, when administered to a subject, will have the intended prophylactic effect, e.g., preventing or delaying the onset (or reoccurrence) a disease, or reducing the likelihood of the onset (or reoccurrence) of a disease or its symptoms. The full prophylactic effect does not necessarily occur by administration of one dose, and may occur only after administration of a series of doses. Thus, a prophylactically effective amount may be administered in one or more administrations. An “activity decreasing amount,” as used herein, refers to an amount of antagonist required to decrease the activity of an enzyme relative to the absence of the antagonist. A “function disrupting amount,” as used herein, refers to the amount of antagonist required to disrupt the function of an osteoclast or leukocyte relative to the absence of the antagonist.

As used herein, the phrase “ATP-binding pocket” refers to the active site of a kinase that binds ATP. The active site of the kinase where ATP binds is the set of amino acid residues that are able to interact with and or bind to an ATP molecule or an ATP competitive inhibitor.

As used herein, the term “mutated” refers to a kinase with a non-natural (e.g. non-wild type) amino acid sequence. A mutated kinase is typically recombinant (e.g. engineered). In some embodiments as described below, the mutated kinase has a cysteine residue substitution at the gatekeeper amino acid position. As used herein, the term “unmutated” refers to the corresponding kinase wherein the mutation (e.g. a cysteine residue is substituted for a gatekeeper amino acid position) is not present (e.g. the natural or wild-type sequence). Thus, in some instances, unmutated refers to the wild-type or natural kinase. In some other instances, the corresponding kinase is another recombinant kinase having similar but distinct substitutions.

As used herein the term “not substantially lower” when referring to  $k_{cat}$  means that the  $k_{cat}$  is not less than a thousandth, i.e.  $1/1000$ , of the corresponding  $k_{cat}$  used for comparison. In some embodiments, the  $k_{cat}$  is not less than a hundredth, i.e.  $1/100$ , of the corresponding  $k_{cat}$  used for comparison. In some instances, the  $k_{cat}$  is not less than a tenth, i.e.  $1/10$ , of the corresponding  $k_{cat}$  used for comparison. In some instances, the  $k_{cat}$  is not less than a quarter, i.e.  $1/4$ , of the corresponding  $k_{cat}$  used for comparison. In some instances, the  $k_{cat}$  is not less than half, i.e.  $1/2$ , of the corresponding  $k_{cat}$  used for comparison. For example, an engineered or mutated kinase may have a  $k_{cat}$  that is not substantially lower than the corresponding  $k_{cat}$  of the corresponding wild-type or natural or unmutated kinase.

As used herein the terms “not substantially lower” when referring to  $K_m$  means that the  $K_m$  is not less than a thousandth, i.e.  $1/1000$ , of the corresponding  $K_m$  used for comparison. In some embodiments, the  $K_m$  is not less than a hundredth, i.e.  $1/100$ , of the corresponding  $K_m$  used for comparison. In some instances, the  $K_m$  is not less than a tenth, i.e.  $1/10$ , of the corresponding  $K_m$  used for comparison. In some instances, the  $K_m$  is not less than a quarter, i.e.  $1/4$ , of the corresponding  $K_m$  used for comparison. In some instances, the  $K_m$  is not less than half, i.e.  $1/2$ , of the corresponding  $K_m$  used for comparison. For example, an engineered or mutated kinase may have a  $K_m$  that is not substantially lower than the corresponding  $K_m$  of the corresponding wild-type or natural or unmutated kinase.

“Disease” or “condition” refer a state of being or health status of a patient or subject capable of being treated with the compounds provided herein. Examples of disorders or conditions include, but are not limited to, cancer, cardiovascular disease, hypertension, Syndrome X, depression, anxiety, glaucoma, human immunodeficiency virus (HIV) or acquired immunodeficiency syndrome (AIDS), neurodegeneration, Alzheimer’s disease, Parkinson’s disease, cognition enhancement, Cushing’s Syndrome, Addison’s Disease, osteoporosis, frailty, muscle frailty, inflammatory diseases, osteoarthritis, rheumatoid arthritis, asthma and rhinitis, adrenal function-related ailments, viral infection, immunodeficiency, immunomodulation, autoimmune diseases, allergies, wound healing, compulsive behavior, multi-drug resistance, addiction, psychosis, anorexia, cachexia, post-traumatic stress syndrome, post-surgical bone fracture, medical catabolism, major psychotic depression, mild cognitive impairment, psychosis, dementia, hyperglycemia, stress disorders, antipsychotic induced weight gain, delirium, cognitive impairment in depressed patients, cognitive deterioration in individuals with Down’s syndrome, psychosis associated with interferon-alpha therapy, chronic pain, pain associated with gastroesophageal reflux disease, postpartum psychosis, postpartum depression, neurological disorders in premature infants, and migraine headaches. In some instances, “disease” or “condition” refer to cancer. In some further instances,

"cancer" refers to human cancers and carcinomas, sarcomas, adenocarcinomas, lymphomas, leukemias, etc., including solid and lymphoid cancers, kidney, breast, lung, bladder, colon, ovarian, prostate, pancreas, stomach, brain, head and neck, skin, uterine, testicular, glioma, esophagus, and liver cancer, including hepatocarcinoma, lymphoma, including B-acute lymphoblastic lymphoma, non-Hodgkin's lymphomas (e.g., Burkitt's, Small Cell, and Large Cell lymphomas) and Hodgkin's lymphoma, leukemia (including AML, ALL, and CML), and multiple myeloma.

As used herein, the term "kinase-associated disease" refers to a disease or condition that is mediated, at least in part, by a kinase.

As used herein, the term "Lrrk-2-associated disease" refers to a disease or condition that is mediated, at least in part, by a Lrrk-2 kinase.

As used herein, the term "cancer" refers to all types of cancer, neoplasm or malignant tumors found in mammals, including leukemia, carcinomas and sarcomas. Exemplary cancers include cancer of the brain, breast, cervix, colon, head & neck, liver, kidney, lung, non-small cell lung, melanoma, mesothelioma, ovary, sarcoma, stomach, uterus and Medulloblastoma. Additional examples include, Hodgkin's Disease, Non-Hodgkin's Lymphoma, multiple myeloma, neuroblastoma, ovarian cancer, rhabdomyosarcoma, primary thrombocytosis, primary macroglobulinemia, primary brain tumors, cancer, malignant pancreatic insulanoma, malignant carcinoid, urinary bladder cancer, premalignant skin lesions, testicular cancer, lymphomas, thyroid cancer, neuroblastoma, esophageal cancer, genitourinary tract cancer, malignant hypercalcemia, endometrial cancer, adrenal cortical cancer, neoplasms of the endocrine and exocrine pancreas, and prostate cancer.

The term "leukemia" refers broadly to progressive, malignant diseases of the blood-forming organs and is generally characterized by a distorted proliferation and development of leukocytes and their precursors in the blood and bone marrow. Leukemia is generally clinically classified on the basis of (1) the duration and character of the disease-acute or chronic; (2) the type of cell involved; myeloid (myelogenous), lymphoid (lymphogenous), or monocytic; and (3) the increase or non-increase in the number abnormal cells in the blood-leukemic or aleukemic (subleukemic). The  $P_{388}$  leukemia model is widely accepted as being predictive of in vivo anti-leukemic activity. It is believed that a compound that tests positive in the  $P_{388}$  assay will generally exhibit some level of anti-leukemic activity in vivo regardless of the type of leukemia being treated. Accordingly, the present invention includes a method of treating leukemia, and, preferably, a method of treating acute nonlymphocytic leukemia, chronic lymphocytic leukemia, acute granulocytic leukemia, chronic granulocytic leukemia, acute promyelocytic leukemia, adult T-cell leukemia, aleukemic leukemia, a leukocythemiac leukemia, basophilic leukemia, blast cell leukemia, bovine leukemia, chronic myelocytic leukemia, leukemia cutis, embryonal leukemia, eosinophilic leukemia, Gross' leukemia, hairy-cell leukemia, hemoblastic leukemia, hemocytoblastic leukemia, histiocytic leukemia, stem cell leukemia, acute monocytic leukemia, leukopenic leukemia, lymphatic leukemia, lymphoblastic leukemia, lymphocytic leukemia, lymphogenous leukemia, lymphoid leukemia, lymphosarcoma cell leukemia, mast cell leukemia, megakaryocytic leukemia, micromyeloblastic leukemia, monocytic leukemia, myeloblastic leukemia, myelocytic leukemia, myeloid granulocytic leukemia, myelomonocytic leukemia, Naegeli leukemia, plasma cell leukemia, multiple myeloma, plasmacytic leukemia, promyelocytic

leukemia, Rieder cell leukemia, Schilling's leukemia, stem cell leukemia, subleukemic leukemia, and undifferentiated cell leukemia.

The term "sarcoma" generally refers to a tumor which is made up of a substance like the embryonic connective tissue and is generally composed of closely packed cells embedded in a fibrillar or homogeneous substance. Sarcomas which can be treated with a combination of antineoplastic thiol-binding mitochondrial oxidant and an anticancer agent include a chondrosarcoma, fibrosarcoma, lymphosarcoma, melanosarcoma, myxosarcoma, osteosarcoma, Abemethy's sarcoma, adipose sarcoma, liposarcoma, alveolar soft part sarcoma, ameloblastic sarcoma, botryoid sarcoma, chloroma sarcoma, chorio carcinoma, embryonal sarcoma, Wilms' tumor sarcoma, endometrial sarcoma, stromal sarcoma, Ewing's sarcoma, fascial sarcoma, fibroblastic sarcoma, giant cell sarcoma, granulocytic sarcoma, Hodgkin's sarcoma, idiopathic multiple pigmented hemorrhagic sarcoma, immunoblastic sarcoma of B cells, lymphoma, immunoblastic sarcoma of T-cells, Jensen's sarcoma, Kaposi's sarcoma, Kupffer cell sarcoma, angiosarcoma, leukosarcoma, malignant mesenchymoma sarcoma, parosteal sarcoma, reticulocytic sarcoma, Rous sarcoma, serocystic sarcoma, synovial sarcoma, and telangiectatic sarcoma.

The term "melanoma" is taken to mean a tumor arising from the melanocytic system of the skin and other organs. Melanomas which can be treated with a combination of antineoplastic thiol-binding mitochondrial oxidant and an anticancer agent include, for example, acral-lentiginous melanoma, amelanotic melanoma, benign juvenile melanoma, Cloudman's melanoma, S91 melanoma, Harding-Passey melanoma, juvenile melanoma, lentigo maligna melanoma, malignant melanoma, nodular melanoma, subungual melanoma, and superficial spreading melanoma.

The term "carcinoma" refers to a malignant new growth made up of epithelial cells tending to infiltrate the surrounding tissues and give rise to metastases. Exemplary carcinomas which can be treated with a combination of antineoplastic thiol-binding mitochondrial oxidant and an anticancer agent include, for example, acinar carcinoma, acinous carcinoma, adenocystic carcinoma, adenoid cystic carcinoma, carcinoma adenomatosum, carcinoma of adrenal cortex, alveolar carcinoma, alveolar cell carcinoma, basal cell carcinoma, carcinoma basocellulare, basaloid carcinoma, basosquamous cell carcinoma, bronchioalveolar carcinoma, bronchiolar carcinoma, bronchogenic carcinoma, cerebriiform carcinoma, cholangiocellular carcinoma, chorionic carcinoma, colloid carcinoma, comedo carcinoma, corpus carcinoma, cribriform carcinoma, carcinoma en cuirasse, carcinoma cutaneum, cylindrical carcinoma, cylindrical cell carcinoma, duct carcinoma, carcinoma durum, embryonal carcinoma, encephaloid carcinoma, epiermoid carcinoma, carcinoma epitheliale adenoides, exophytic carcinoma, carcinoma ex ulcere, carcinoma fibrosum, gelatiniform carcinoma, gelatinous carcinoma, giant cell carcinoma, carcinoma gigantocellulare, glandular carcinoma, granulosa cell carcinoma, hair-matrix carcinoma, hematoid carcinoma, hepatocellular carcinoma, Hurtle cell carcinoma, hyaline carcinoma, hypemephrad carcinoma, infantile embryonal carcinoma, carcinoma in situ, intraepidermal carcinoma, intraepithelial carcinoma, Krompecher's carcinoma, Kulchitzky-cell carcinoma, large-cell carcinoma, lenticular carcinoma, carcinoma lenticulare, lipomatous carcinoma, lymphoepithelial carcinoma, carcinoma medullare, medullary carcinoma, melanotic carcinoma, carcinoma molle, mucinous carcinoma, carcinoma muciparum, carcinoma mucocellulare, mucoepidermoid carcinoma, carcinoma mucosum, mucous carcinoma, carcinoma

myxomatodes, nasopharyngeal carcinoma, oat cell carcinoma, carcinoma ossificans, osteoid carcinoma, papillary carcinoma, periportal carcinoma, preinvasive carcinoma, prickle cell carcinoma, pultaceous carcinoma, renal cell carcinoma of kidney, reserve cell carcinoma, carcinoma sarcomatodes, schneiderian carcinoma, scirrhous carcinoma, carcinoma scroti, signet-ring cell carcinoma, carcinoma simplex, small-cell carcinoma, solanoid carcinoma, spheroidal cell carcinoma, spindle cell carcinoma, carcinoma spongiosum, squamous carcinoma, squamous cell carcinoma, string carcinoma, carcinoma telangiectaticum, carcinoma telangiectodes, transitional cell carcinoma, carcinoma tuberosum, tuberosus carcinoma, verrucous carcinoma, and carcinoma villosum.

The term "pharmaceutically acceptable salts" is meant to include salts of the active compounds which are prepared with relatively nontoxic acids or bases, depending on the particular substituents found on the compounds described herein. When compounds of the present invention contain relatively acidic functionalities, base addition salts can be obtained by contacting the neutral form of such compounds with a sufficient amount of the desired base, either neat or in a suitable inert solvent. Examples of pharmaceutically acceptable base addition salts include sodium, potassium, calcium, ammonium, organic amino, or magnesium salt, or a similar salt. When compounds of the present invention contain relatively basic functionalities, acid addition salts can be obtained by contacting the neutral form of such compounds with a sufficient amount of the desired acid, either neat or in a suitable inert solvent. Examples of pharmaceutically acceptable acid addition salts include those derived from inorganic acids like hydrochloric, hydrobromic, nitric, carbonic, monohydrogencarbonic, phosphoric, monohydrogenphosphoric, dihydrogenphosphoric, sulfuric, monohydrogensulfuric, hydriodic, or phosphorous acids and the like, as well as the salts derived from relatively nontoxic organic acids like acetic, propionic, isobutyric, maleic, malonic, benzoic, succinic, suberic, fumaric, lactic, mandelic, phthalic, benzenesulfonic, p-tolylsulfonic, citric, tartaric, methanesulfonic, and the like. Also included are salts of amino acids such as arginate and the like, and salts of organic acids like glucuronic or galactunoric acids and the like (see, for example, Berge et al., "Pharmaceutical Salts", *Journal of Pharmaceutical Science*, 1977, 66, 1-19). Certain specific compounds of the present invention contain both basic and acidic functionalities that allow the compounds to be converted into either base or acid addition salts.

The neutral forms of the compounds are preferably regenerated by contacting the salt with a base or acid and isolating the parent compound in the conventional manner. The parent form of the compound differs from the various salt forms in certain physical properties, such as solubility in polar solvents.

In addition to salt forms, the present invention provides compounds, which are in a prodrug form. Prodrugs of the compounds described herein are those compounds that readily undergo chemical changes under physiological conditions to provide the compounds of the present invention. Additionally, prodrugs can be converted to the compounds of the present invention by chemical or biochemical methods in an ex vivo environment. For example, prodrugs can be slowly

converted to the compounds of the present invention when placed in a transdermal patch reservoir with a suitable enzyme or chemical reagent.

Certain compounds of the present invention can exist in unsolvated forms as well as solvated forms, including hydrated forms. In general, the solvated forms are equivalent to unsolvated forms and are encompassed within the scope of the present invention. Certain compounds of the present invention may exist in multiple crystalline or amorphous forms. In general, all physical forms are equivalent for the uses contemplated by the present invention and are intended to be within the scope of the present invention.

Certain compounds of the present invention possess asymmetric carbon atoms (optical centers) or double bonds; the racemates, diastereomers, geometric isomers and individual isomers are encompassed within the scope of the present invention.

The compounds of the present invention may also contain unnatural proportions of atomic isotopes at one or more of the atoms that constitute such compounds. For example, the compounds may be radiolabeled with radioactive isotopes, such as for example tritium ( $^3\text{H}$ ), iodine-125 ( $^{125}\text{I}$ ) or carbon-14 ( $^{14}\text{C}$ ). All isotopic variations of the compounds of the present invention, whether radioactive or not, are encompassed within the scope of the present invention.

As used herein, the term "salt" refers to acid or base salts of the compounds used in the methods of the present invention. Illustrative examples of acceptable salts are mineral acid (hydrochloric acid, hydrobromic acid, phosphoric acid, and the like) salts, organic acid (acetic acid, propionic acid, glutamic acid, citric acid and the like) salts, quaternary ammonium (methyl iodide, ethyl iodide, and the like) salts.

As used herein, the term "isomers" refers to compounds having the same number and kind of atoms, and hence the same molecular weight, but differing in respect to the structural arrangement or configuration of the atoms.

As used herein, the term "tautomer," refers to one of two or more structural isomers which exist in equilibrium and which are readily converted from one isomeric form to another.

### III. Introduction

Provided herein are, inter alia, novel methods and compositions for inhibiting a protein kinase, e.g., a cysteine substituted kinase, determining the function of a protein kinase in a cell, and treating kinase-associated diseases and conditions. Certain heterocyclic compounds having an electrophilic substituent provided herein that specifically, and optionally irreversibly, inhibit cysteine substituted kinases. In some embodiments, the heterocyclic compound comprises two or more fused rings and an electrophilic substituent. In some embodiments, at least one of the two or more fused rings comprises a nitrogen atom. In some embodiments, the heterocyclic compounds inhibit a cysteine substituted kinase, i.e., a kinase having a cysteine residue in the gatekeeper position of the ATP binding site. In some embodiments, the heterocyclic compounds also inhibit a kinase not having a cysteine residue in the gatekeeper position (e.g. of the ATP binding site).

### IV. Compounds

The present invention provides compounds suitable for use with the methods and assays described herein.

In some other embodiments, the heterocyclic compounds useful for inhibiting a kinase include two or more fused rings which include at least one heteroatom selected from N, O, or S. In some embodiments, the fused rings are substituted with a ring selected from substituted or unsubstituted cycloalkyl, substituted or unsubstituted aryl, or substituted or unsubstituted

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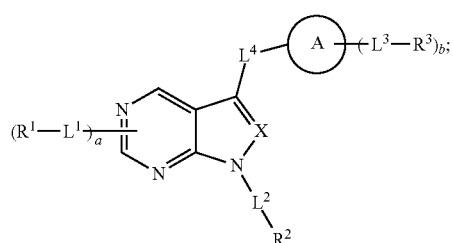
tuted heteroaryl. In some other embodiments, the cycloalkyl, heterocycloalkyl, aryl, or heteroaryl is substituted with a substituent selected from substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl. In other embodiments, the ring which substitutes the fused rings is an aryl or heteroaryl. In some embodiments, the ring which substitutes the fused rings is an aryl. In some other embodiments, the ring which substitutes the fused rings is an aryl which is substituted with an electrophilic substituent that is capable of accepting electron density from a cysteine gatekeeper residue of a protein kinase. In some embodiments, the electrophilic substituent is capable of forming a covalent bond to the sulfhydryl group of the cysteine gate keeper residue.

In some embodiments, the compound is a substituted or unsubstituted phenyl-derivatized pyrazolopyrimidine, e.g. 3-phenyl-substituted pyrazolopyrimidines, having an electrophilic substituent. In some other embodiments, the present invention provides 3-phenyl-substituted pyrazolopyrimidines which are synthesized with an electrophilic groups at positions expected to be in close proximity to the gatekeeper residue. In some embodiments, compound is a substituted or unsubstituted quinazoline having an electrophilic substituent. In some embodiments, compound is a substituted or unsubstituted 4-anilinoquinazoline, e.g. Michael acceptor-derivatized 4-anilinoquinazolines, having an electrophilic substituent. In some embodiments, the compound is a substituted or unsubstituted benzyl-derivatized pyrazolopyrimidine having an electrophilic substituent. In some embodiments, the compound is a substituted or unsubstituted pyrazolopyrimidine having an electrophilic substituent pyrazolopyrimidine.

In some other embodiments, the electrophilic substituent is an electrophilic ATP-binding pocket moiety (i.e. a chemical moiety that interacts with amino acids that form part of the ATP-binding pocket). In other embodiments, the electrophilic substituent is a vinylsulfonamide, a vinylsulfone, an acrylamide, a chloroacetamide, an  $\alpha$ -chloroacetamide, an epoxide, or a fluoromethylketones.

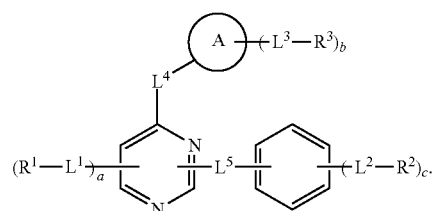
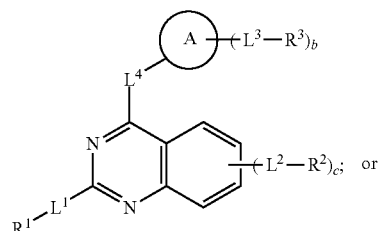
In some embodiments the compounds described herein are inhibitors of kinases ("kinase inhibitors") such as an inhibitor of a recombinant cysteine gatekeeper kinase ("cysteine gatekeeper kinase inhibitor"). In some other embodiments, the cysteine gatekeeper kinase inhibitor includes an ATP-binding pocket moiety (e.g. an ATP-binding pocket moiety including a heterocyclic moiety) covalently bound to an electrophilic moiety capable of binding the thiol of the gatekeeper cysteine residue of the cysteine gatekeeper kinase. In some embodiments, the inhibitor is one or more of the compounds set forth in Table 1 of Formulas (I) to (XXIX) (e.g. Formula (I) to (XIV)).

In some embodiments, the compounds has the formula:



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-continued



In these formula, X is  $=N-$  or  $=C(-L^6-R^6)-$ ; Ring A is, in each instance, independently cycloalkyl, heterocycloalkyl, aryl, or heteroaryl;  $L^1$ ,  $L^2$ ,  $L^3$ ,  $L^4$ ,  $L^5$ , and  $L^6$  are, in each instance, independently selected from a bond,  $-C(O)-$ ,  $-C(O)N(R^7)-$ ,  $-C(O)O-$ ,  $-S(O)_g-$  (i.e.  $-S-$ ,  $-S(O)-$  or  $-S(O)_2-$ ),  $-S(O)_2N(R^7)-$ ,  $-O-$ ,  $-N(R^7)-$ ,  $-N(R^7)C(O)N(R^8)-$ , substituted or unsubstituted alkylene, substituted or unsubstituted heteroalkylene, substituted or unsubstituted cycloalkylene, substituted or unsubstituted heterocycloalkylene, substituted or unsubstituted arylene, or substituted or unsubstituted heteroarylene, wherein g is independently an integer from 0 to 2;  $R^1$ ,  $R^2$ ,  $R^3$ ,  $R^4$ ,  $R^5$ ,  $R^6$ ,  $R^7$ , and  $R^8$  are, in each instance, independently selected from hydrogen, halogen,  $-CN$ ,  $-OH$ ,  $-NH_2$ ,  $-COOH$ ,  $-CONH_2$ ,  $-NO_2$ ,  $-SH$ ,  $-SO_2Cl$ ,  $-SO_3H$ ,  $-SO_4H$ ,  $-SO_2NH_2$ , substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl; a is an integer from 0 to 2; b is an integer from 0 to 5; and c is an integer from 0 to 4. In some embodiments, A is aryl (e.g. phenyl). In some embodiments,  $R^7$  is hydrogen. In some embodiments,  $R^7$  and  $R^8$  are hydrogen.  $R^1$ ,  $R^2$ ,  $R^3$ ,  $R^4$ ,  $R^5$ ,  $R^6$ ,  $R^7$ , and  $R^8$  may also independently be hydrogen, halogen (e.g.  $-Cl$  or  $-F$ ),  $-CN$ ,  $-OH$ ,  $-NH_2$ ,  $-COOH$ ,  $-CONH_2$ ,  $-NO_2$ ,  $-SH$ , substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl.

In some embodiments,  $R^1$  is hydrogen, halogen,  $-CN$ ,  $-OH$ ,  $-NH_2$ ,  $-COOH$ ,  $-CONH_2$ ,  $-NO_2$ ,  $-SH$ ,  $-SO_2Cl$ ,  $-SO_3H$ ,  $-SO_4H$ ,  $-SO_2NH_2$ ,  $R^9$ -substituted or unsubstituted alkyl,  $R^9$ -substituted or unsubstituted heteroalkyl,  $R^9$ -substituted or unsubstituted cycloalkyl,  $R^9$ -substituted or unsubstituted heterocycloalkyl,  $R^9$ -substituted or unsubstituted aryl, or  $R^9$ -substituted or unsubstituted heteroaryl.

$R^9$  is independently halogen,  $-CN$ ,  $-OH$ ,  $-NH_2$ ,  $-COOH$ ,  $-CONH_2$ ,  $-NO_2$ ,  $-SH$ ,  $-SO_2Cl$ ,  $-SO_3H$ ,  $-SO_4H$ ,  $-SO_2NH_2$ ,  $R^{10}$ -substituted or unsubstituted alkyl,  $R^{10}$ -substituted or unsubstituted heteroalkyl,  $R^{10}$ -substituted or unsubstituted cycloalkyl,  $R^{10}$ -substituted or unsubstituted heterocycloalkyl,  $R^{10}$ -substituted or unsubstituted aryl, or  $R^{10}$ -substituted or unsubstituted heteroaryl.

R<sup>27</sup> is independently halogen, —CN, —OH, —NH<sub>2</sub>, —COOH, —CONH<sub>2</sub>, —NO<sub>2</sub>, —SH, —SO<sub>2</sub>Cl, —SO<sub>3</sub>H, —SO<sub>4</sub>H, —SO<sub>2</sub>NH<sub>2</sub>, R<sup>28</sup>-substituted or unsubstituted alkyl, R<sup>28</sup>-substituted or unsubstituted heteroalkyl, R<sup>28</sup>-substituted or unsubstituted cycloalkyl, R<sup>28</sup>-substituted or unsubstituted heterocycloalkyl, R<sup>28</sup>-substituted or unsubstituted aryl, or R<sup>28</sup>-substituted or unsubstituted heteroarvl.

In some embodiments,  $L^5$  is in each instance, indepen- 65 dently selected from a bond,  $-\text{C}(\text{O})-$ ,  $-\text{C}(\text{O})\text{N}(\text{R}^7)-$ ,  $-\text{C}(\text{O})\text{O}-$ ,  $-\text{S}(\text{O})_g-$  (i.e.  $-\text{S}-$ ,  $-\text{S}(\text{O})-$  or  $-\text{S}(\text{O})_2-$ ),  $-\text{S}(\text{O})_g\text{N}(\text{R}^7)-$ ,  $-\text{O}-$ ,  $-\text{N}(\text{R}^7)-$ ,  $-\text{N}(\text{R}^7)\text{C}(\text{O})\text{N}-$

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(R<sup>8</sup>)—, R<sup>45</sup>-substituted or unsubstituted alkylene, R<sup>45</sup>-substituted or unsubstituted heteroalkylene, R<sup>45</sup>-substituted or unsubstituted cycloalkylene, R<sup>45</sup>-substituted or unsubstituted heterocycloalkylene, R<sup>45</sup>-substituted or unsubstituted arylene, or R<sup>45</sup>-substituted or unsubstituted heteroarylene.

R<sup>45</sup> is independently halogen, —CN, —OH, —NH<sub>2</sub>, —COOH, —CONH<sub>2</sub>, —NO<sub>2</sub>, —SH, —SO<sub>2</sub>Cl, —SO<sub>3</sub>H, —SO<sub>4</sub>H, —SO<sub>2</sub>NH<sub>2</sub>, R<sup>46</sup>-substituted or unsubstituted alkyl, R<sup>46</sup>-substituted or unsubstituted heteroalkyl, R<sup>46</sup>-substituted or unsubstituted cycloalkyl, R<sup>46</sup>-substituted or unsubstituted heterocycloalkyl, R<sup>46</sup>-substituted or unsubstituted aryl, or R<sup>46</sup>-substituted or unsubstituted heteroaryl.

R<sup>46</sup> is independently halogen, —CN, —OH, —NH<sub>2</sub>, —COOH, —CONH<sub>2</sub>, —NO<sub>2</sub>, —SH, —SO<sub>2</sub>Cl, —SO<sub>3</sub>H, —SO<sub>4</sub>H, —SO<sub>2</sub>NH<sub>2</sub>, R<sup>47</sup>-substituted or unsubstituted alkyl, R<sup>47</sup>-substituted or unsubstituted heteroalkyl, R<sup>47</sup>-substituted or unsubstituted cycloalkyl, R<sup>47</sup>-substituted or unsubstituted heterocycloalkyl, R<sup>47</sup>-substituted or unsubstituted aryl, or R<sup>47</sup>-substituted or unsubstituted heteroaryl.

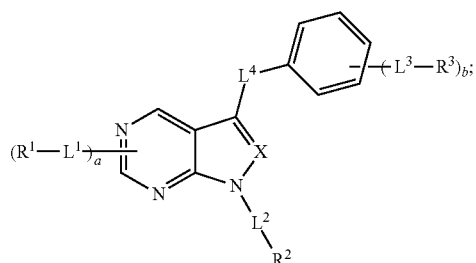
In some embodiments, L<sup>6</sup> is in each instance, independently selected from a bond, —C(O)—, —C(O)N(R<sup>7</sup>)—, —C(O)O—, —S(O)<sub>g</sub>— (i.e. —S—, —S(O)— or —S(O)<sub>2</sub>—), —S(O)<sub>2</sub>N(R<sup>7</sup>)—, —O—, —N(R<sup>7</sup>)—, —N(R<sup>7</sup>)C(O)N(R<sup>8</sup>)—, R<sup>48</sup>-substituted or unsubstituted alkylene, R<sup>48</sup>-substituted or unsubstituted heteroalkylene, R<sup>48</sup>-substituted or unsubstituted cycloalkylene, R<sup>48</sup>-substituted or unsubstituted heterocycloalkylene, R<sup>48</sup>-substituted or unsubstituted arylene, or R<sup>48</sup>-substituted or unsubstituted heteroarylene.

R<sup>48</sup> is independently halogen, —CN, —OH, —NH<sub>2</sub>, —COOH, —CONH<sub>2</sub>, —NO<sub>2</sub>, —SH, —SO<sub>2</sub>Cl, —SO<sub>3</sub>H, —SO<sub>4</sub>H, —SO<sub>2</sub>NH<sub>2</sub>, R<sup>49</sup>-substituted or unsubstituted alkyl, R<sup>49</sup>-substituted or unsubstituted heteroalkyl, R<sup>49</sup>-substituted or unsubstituted cycloalkyl, R<sup>49</sup>-substituted or unsubstituted heterocycloalkyl, R<sup>49</sup>-substituted or unsubstituted aryl, or R<sup>49</sup>-substituted or unsubstituted heteroaryl.

R<sup>49</sup> is independently halogen, —CN, —OH, —NH<sub>2</sub>, —COOH, —CONH<sub>2</sub>, —NO<sub>2</sub>, —SH, —SO<sub>2</sub>Cl, —SO<sub>3</sub>H, —SO<sub>4</sub>H, —SO<sub>2</sub>NH<sub>2</sub>, R<sup>50</sup>-substituted or unsubstituted alkyl, R<sup>50</sup>-substituted or unsubstituted heteroalkyl, R<sup>50</sup>-substituted or unsubstituted cycloalkyl, R<sup>50</sup>-substituted or unsubstituted heterocycloalkyl, R<sup>50</sup>-substituted or unsubstituted aryl, or R<sup>50</sup>-substituted or unsubstituted heteroaryl.

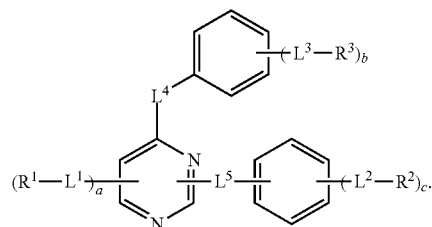
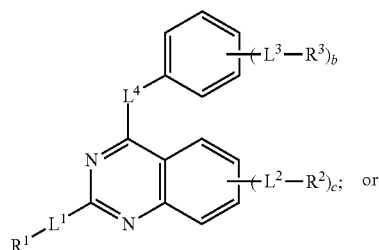
In some embodiments, R<sup>35</sup>, R<sup>38</sup>, R<sup>41</sup>, R<sup>44</sup>, R<sup>47</sup> and R<sup>50</sup> are independently hydrogen, unsubstituted alkyl, unsubstituted heteroalkyl, unsubstituted cycloalkyl, unsubstituted heterocycloalkyl, unsubstituted aryl, or unsubstituted heteroaryl.

In some other embodiments, the compounds have the formula (where the variables are as described above):



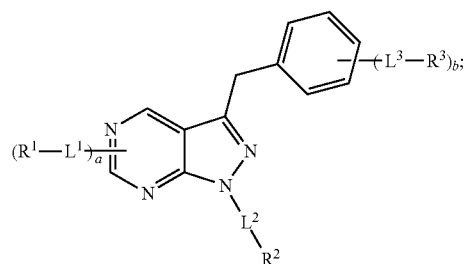
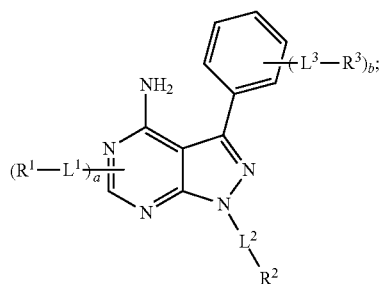
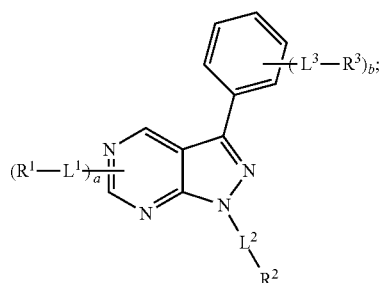
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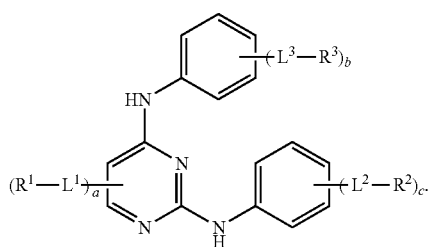
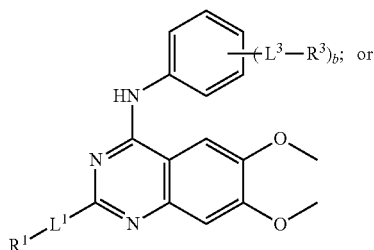
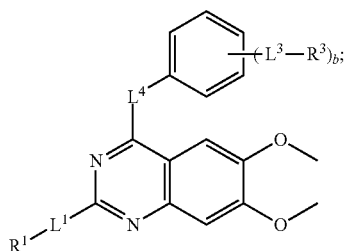
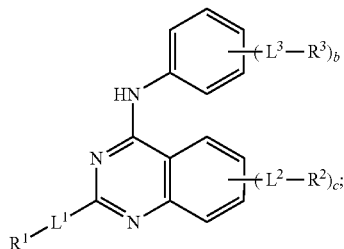
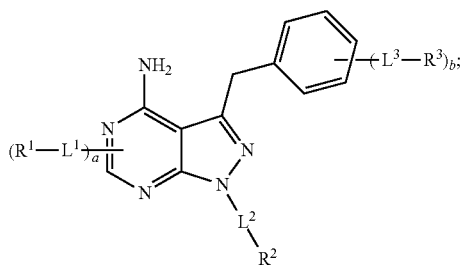
In certain embodiments, L<sup>1</sup>, L<sup>2</sup>, L<sup>3</sup>, L<sup>4</sup>, L<sup>5</sup>, and L<sup>6</sup> are, in each instance, independently a bond, —NH—, or substituted or unsubstituted C<sub>1</sub>-C<sub>5</sub> alkylene. In certain other embodiments, L<sup>6</sup> is a bond, —NH—, or unsubstituted C<sub>1</sub>-C<sub>5</sub> alkylene.

In some other embodiments, the compounds have the formula (where the variables are as described above):



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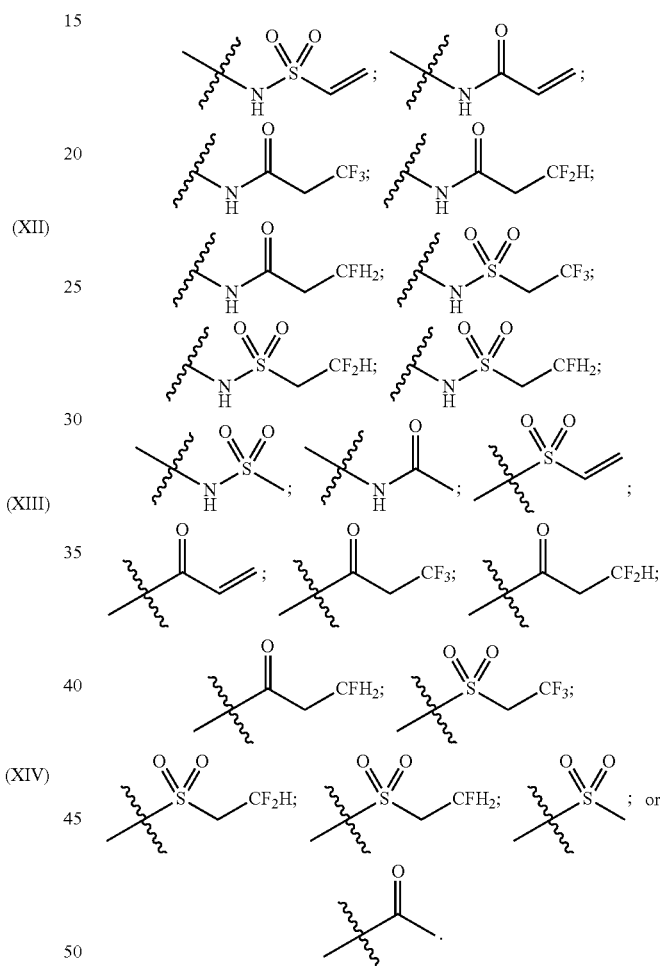


In certain embodiments, at least one  $-L^3-R^3$  is an electrophilic moiety (e.g.  $-L^3-R^3$  is or includes an electrophilic moiety). For example, in some embodiments,  $-L^3-R^3$  forms an electrophilic moiety. In other embodiments, one of  $L^3$  or  $R^3$  is an electrophilic moiety (e.g. one of  $L^3$  or  $R^3$  is or includes an electrophilic moiety). In some embodiments,  $L^3$  forms an electrophilic moiety. In other embodiments,  $R^3$  forms an electrophilic moiety. In certain other embodiments,  $L^3$  is a bond,  $-NH-$ , substituted or unsubstituted alkylene, or substituted or unsubstituted heteroalkylene; and  $R^3$  is a substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, or halogen. In some of the embodiments,  $L^3$  is  $-C(O)-$ ,

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(X)  $-S(O)_2-$ ,  $-NHC(O)-$ , or  $-NHS(O)_2-$ .  $R^3$  may be a substituted or unsubstituted alkyl (e.g. substituted or unsubstituted  $C_1$  to  $C_5$  alkyl). For example,  $R^3$  may be an unsubstituted alkyl or alkyl substituted with chloro, fluoro, methyl, difluoromethyl, or trifluoromethyl. In other embodiments,  $R^3$  is ethenyl, ethyl, 2,2,2-trichloroethyl, 2,2-dichloroethyl, 2-chloroethyl, 2,2,2-trifluoroethyl, 2,2-difluoroethyl, or 2-fluoroethyl, propyl, isopropyl, 1-propenyl, or 2-propenyl.

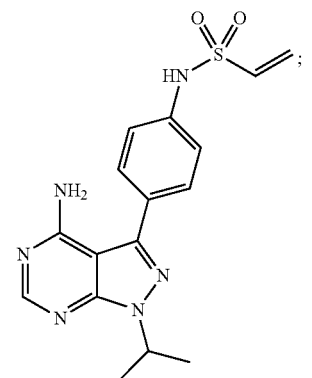
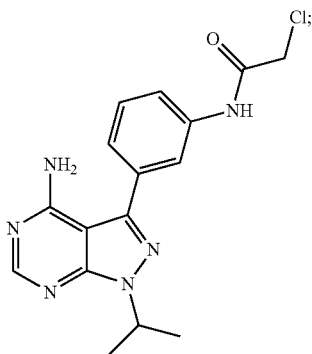
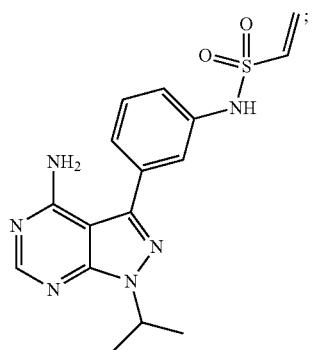
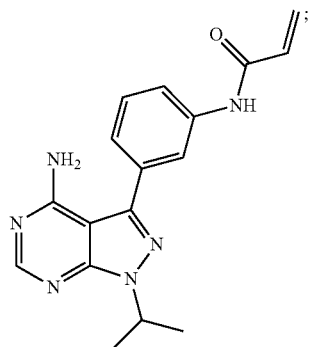
(XI) In some embodiments, the compounds suitable for use with the present invention have the structure of one of the formula cited herein wherein  $-L^3-R^3$  is:



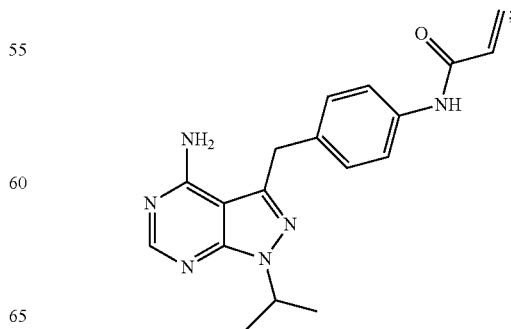
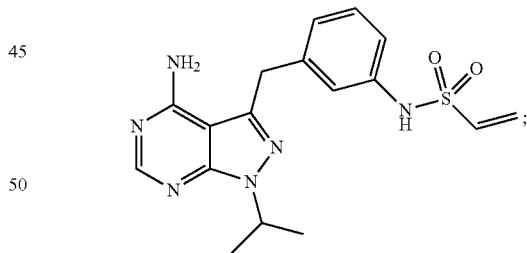
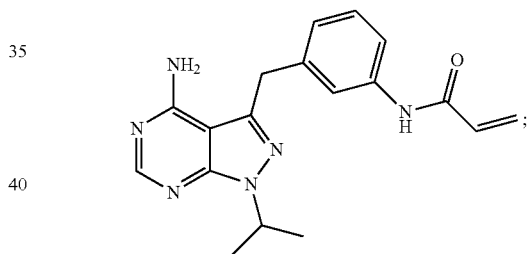
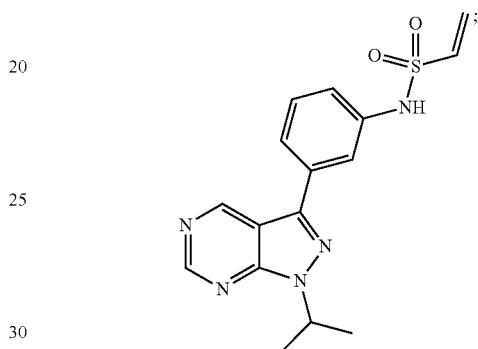
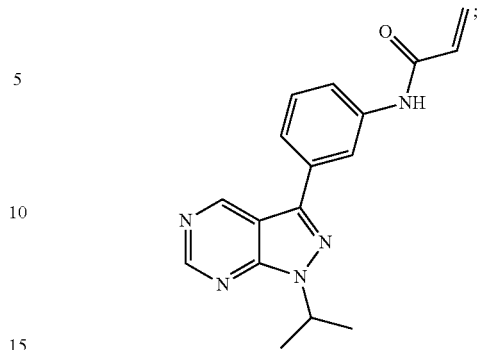
In some other embodiments, the compounds suitable for use with the present invention include those wherein  $L^1$  is a bond.  $R^1$  may be hydrogen or  $NH_2$ . In certain embodiments,  $L^1$  is a bond; and  $R^1$  is hydrogen. In certain other embodiments,  $L^1$  is a bond; and  $R^1$  is  $NH_2$ . In some embodiments, the present invention provides a compound where  $L^2$  is a bond; and  $R^2$  is methyl, ethyl, propyl, isopropyl, butyl, tert-butyl, pentyl, cyclopentyl, hexyl, cyclohexyl, methoxy, ethoxy, propoxy, or butoxy. In certain embodiments,  $R^2$  is isopropyl or cyclopentyl. In other embodiments,  $R^2$  is isopropyl. In other embodiments,  $R^2$  is cyclopentyl. In some other embodiments,  $R^2$  is methoxy. In certain embodiments,  $c$  is 2;  $L^2$  is a bond, and  $R^2$  is methoxy, ethoxy, propoxy, or butoxy. In other embodiments,  $R^2$  is methoxy.

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In some embodiments, the present invention provides compounds that have:

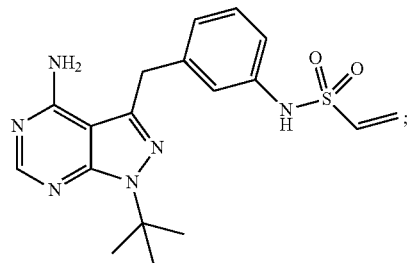
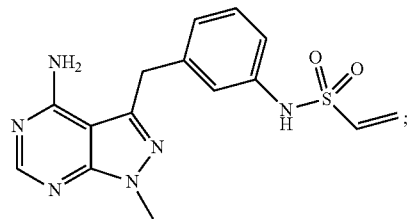
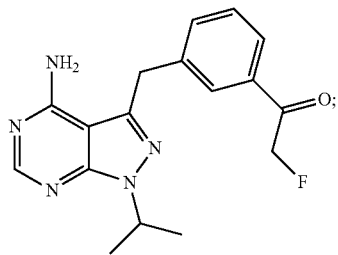
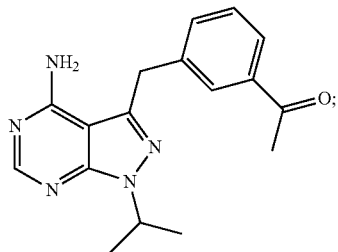
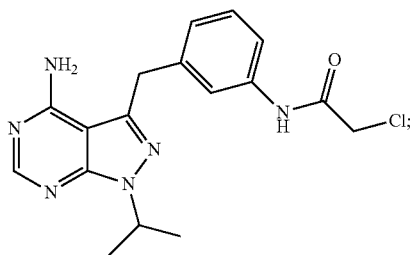
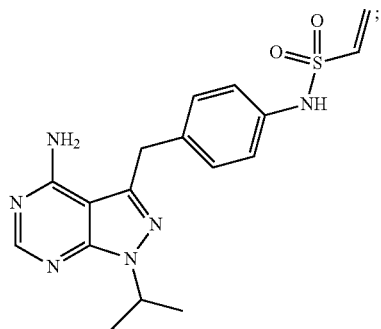
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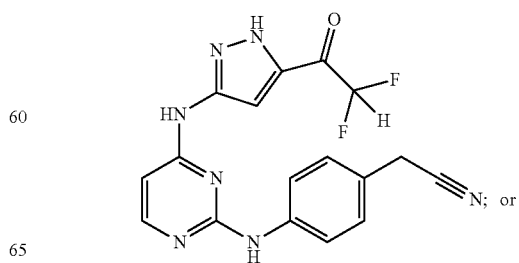
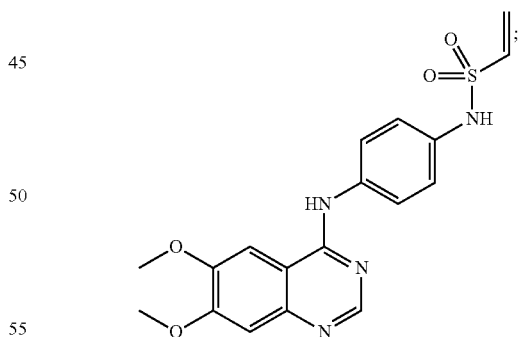
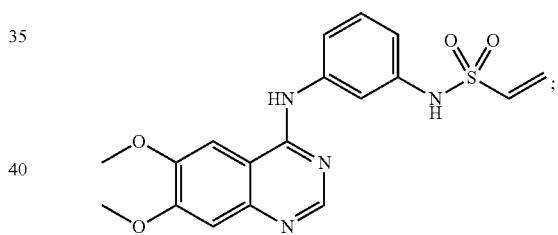
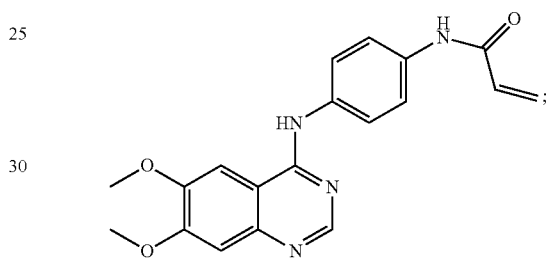
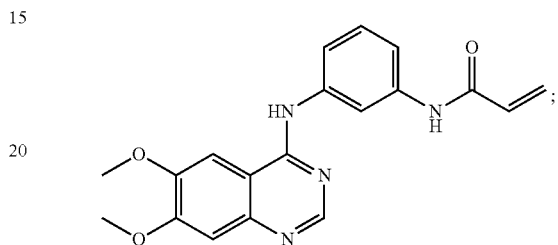
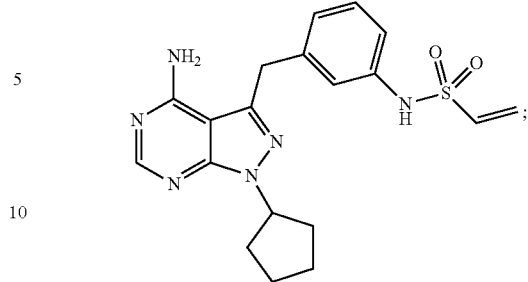


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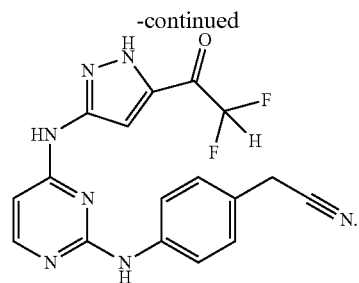
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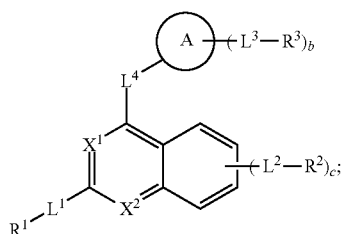
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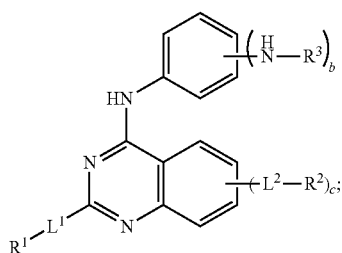


In some other embodiments, the present invention provides a compound having the below formula (which are useful inter alia, as inhibitors of Lrrk-2 kinases):



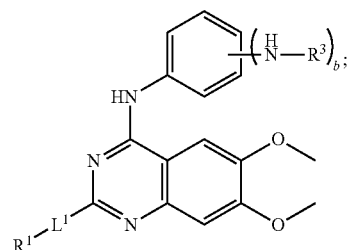
X¹ and X² are, in each instance, independently =N— or =C(—L⁶—R⁶)—. Ring A is, in each instance, independently selected from cycloalkyl, heterocycloalkyl, aryl, or heteroaryl. L¹, L², L³, and L⁴ are as defined above (e.g., in each instance, independently selected from a bond, —C(O)—, —C(O)N(R⁷)—, —C(O)O—, —S(O)ᵍ—, —S(O)₂N(R⁷)—, —O—, —N(R⁷)—, —N(R⁷)C(O)N(R⁸)—), substituted or unsubstituted alkylene, substituted or unsubstituted heteroalkylene, substituted or unsubstituted cycloalkylene, substituted or unsubstituted heterocycloalkylene, substituted or unsubstituted arylene, or substituted or unsubstituted heteroarylene, wherein g is an integer from 0 to 2). R¹, R², R³, R⁶, R⁷, and R⁸ are as defined above (e.g., in each instance, independently selected from hydrogen, halogen, —CN, —OH, —NH₂, —COOH, —CONH₂, —NO₂, —SH, —SO₂Cl, —SO₃H, —SO₄H, —SO₂NH₂, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl). The variable b is as defined above (e.g. an integer from 0 to 5; and c is as defined above (e.g. an integer from 0 to 4).

In some embodiments, the present invention provides a compound having the formula:

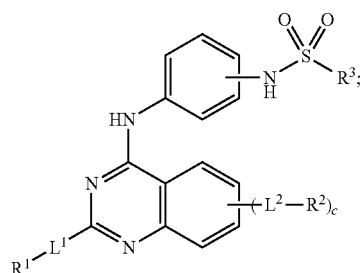


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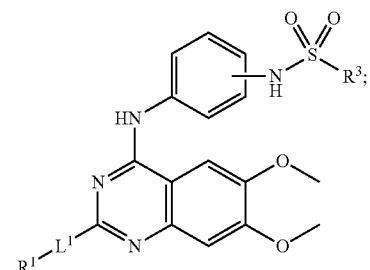
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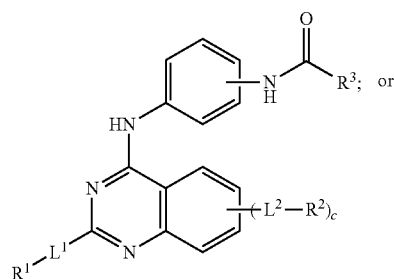
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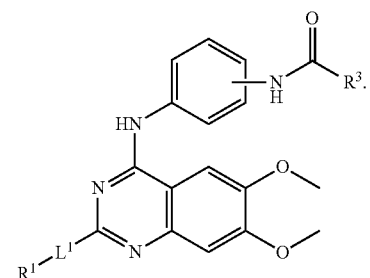
(XVIII)



(XIX)

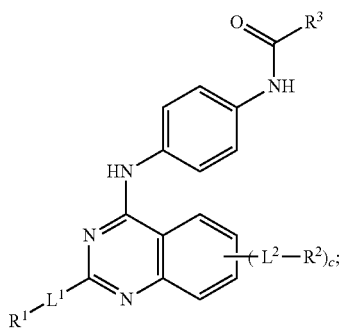
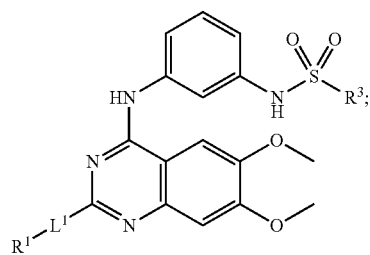
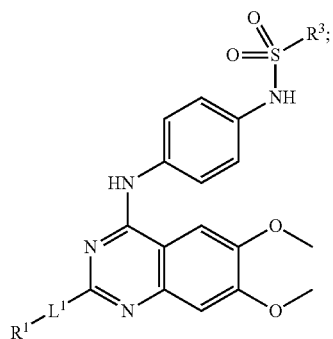
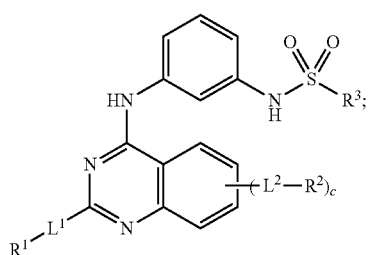
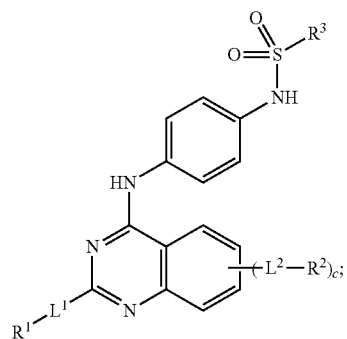


(XX)



(XXI)

In some other embodiments, the compound provided herein has the formula (with the variables as defined above):

**43****44**

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(XXII)

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(XXIII)

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(XXIV)

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(XXV)

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(XXVI)

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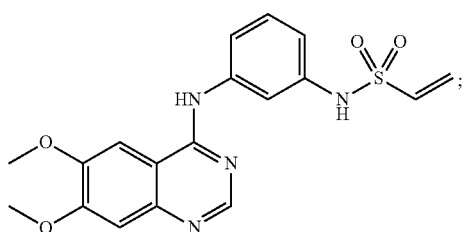
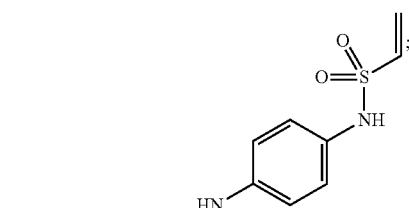
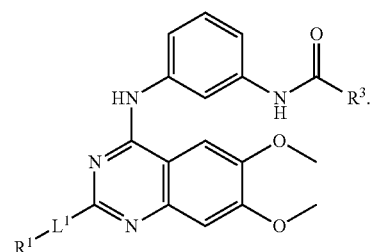
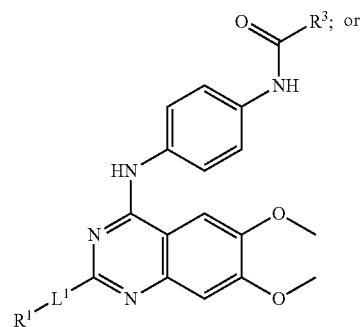
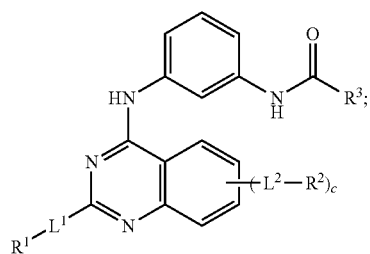
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(XXVII)

(XXVIII)

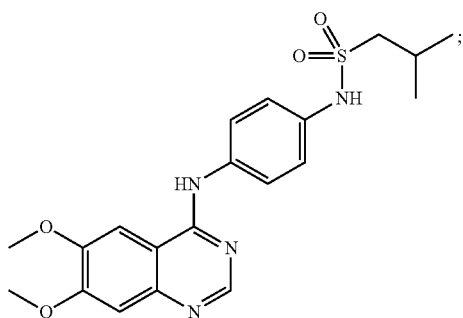
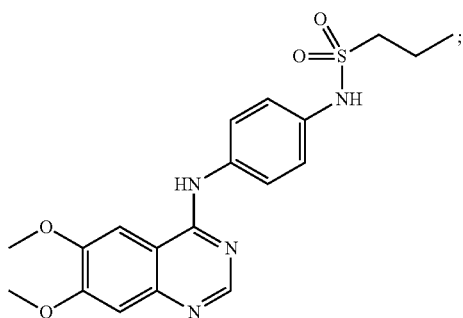
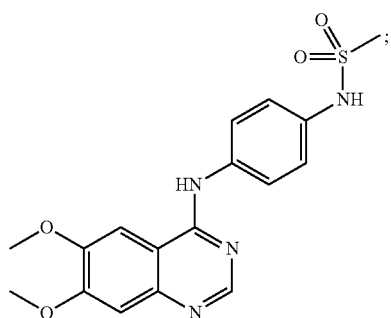
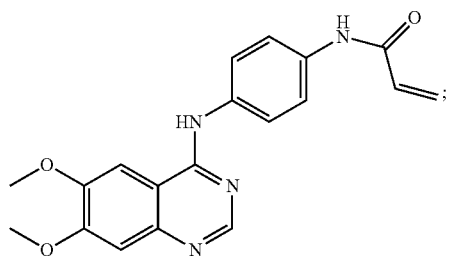
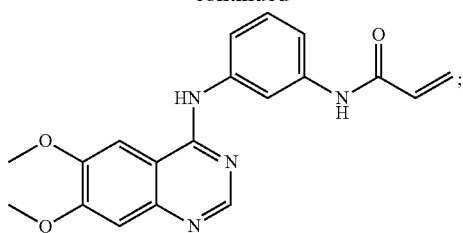
(XXIX)

In other embodiments, the present invention provides compounds having the formula in the table below ("Table 1"):

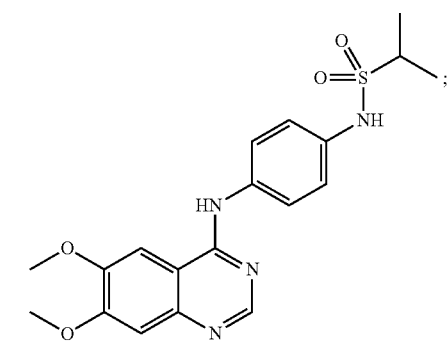
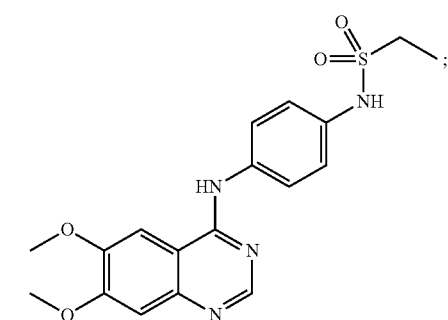
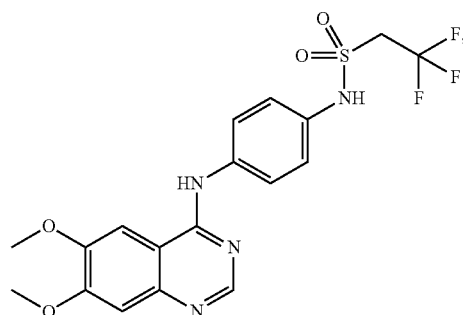
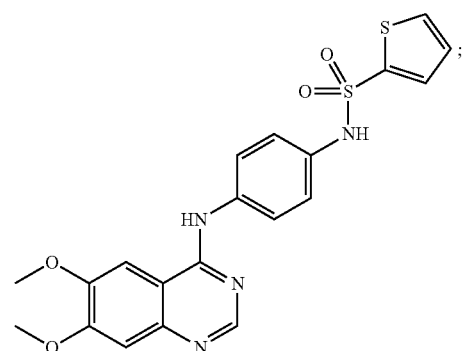
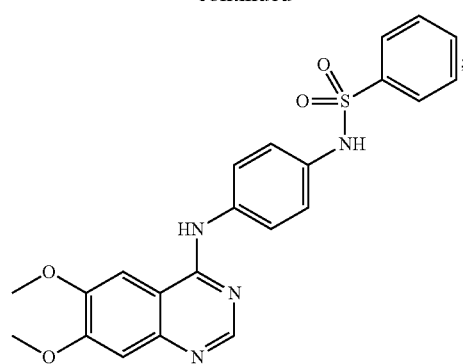


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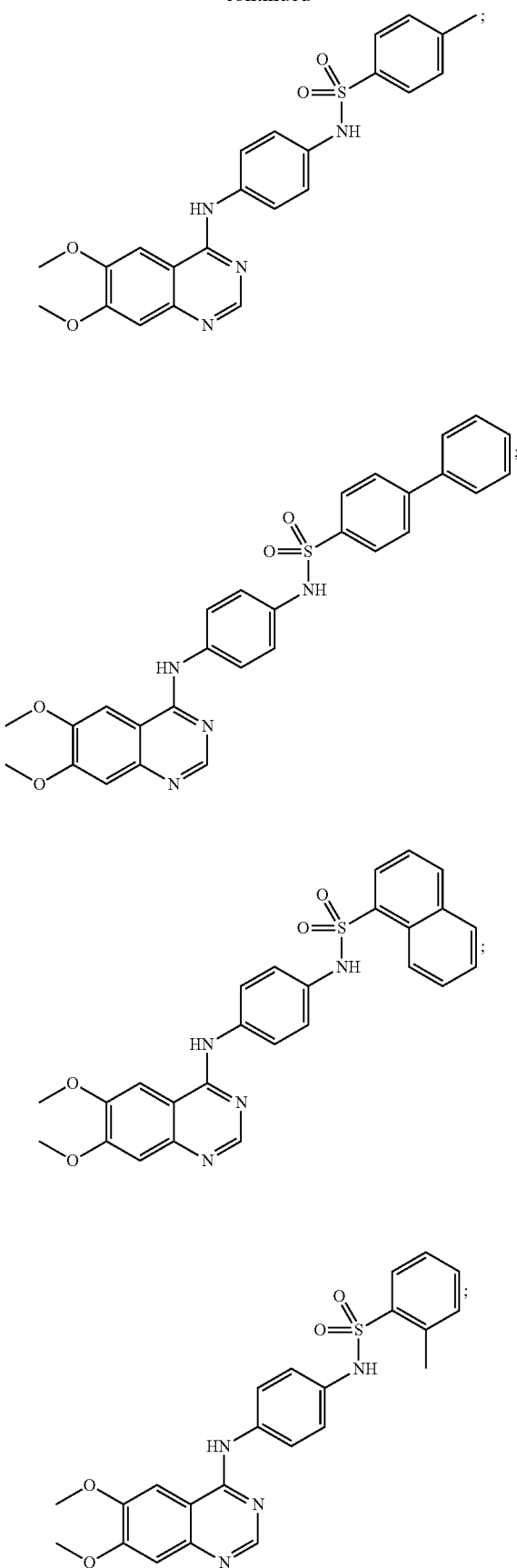
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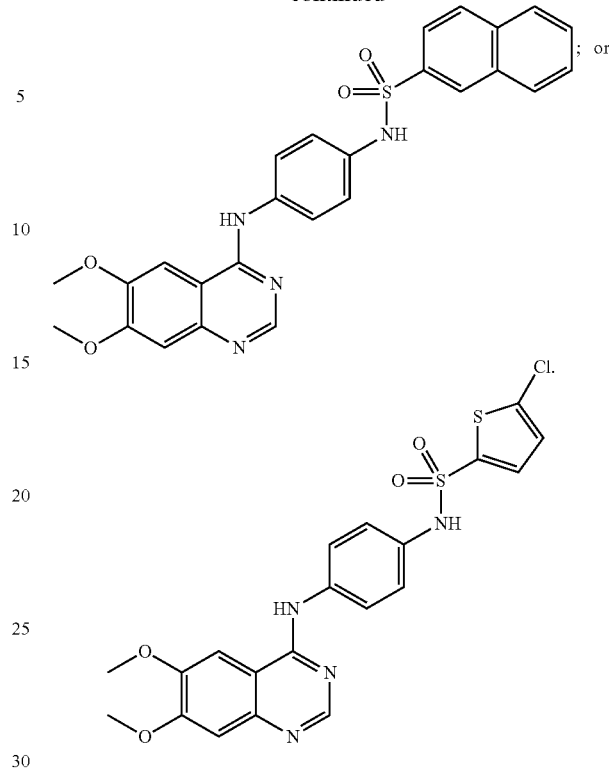
47

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In certain embodiments,  $L^3$  is selected from a bond,  $-\text{NH}-$ ,  $-\text{C}(\text{O})-$ ,  $-\text{S}(\text{O}_2)-$ , substituted or unsubstituted alkylene, or substituted or unsubstituted heteroalkylene.  $R^3$  may be selected from substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, or halogen. In other embodiments,  $R^3$  is methyl; difluoromethyl; trifluoromethyl; ethenyl; ethyl; 2,2,2-trichloroethyl; 2,2-dichloroethyl; 2-chloroethyl; 2,2,2-trifluoroethyl; 2,2-difluoroethyl; or 2-fluoroethyl; propyl; isopropyl; 1-propenyl; 2-propenyl; butyl; tert-butyl; naphthyl; thiophene; 2-chloro-thiophene; phenyl; 2-methyl-phenyl; 3-methyl-phenyl; 4-methyl-phenyl; 2-phenyl-phenyl; 3-phenyl-phenyl; 4-phenyl-phenyl 2-chloro-thiophene; or 3-chloro-thiophene.

As described above, the term “inhibitor” may refer to an inhibitor of a recombinant kinase comprising a cysteine substitution at a gatekeeper amino acid position (i.e. a cysteine gatekeeper kinase inhibitor) and includes a compound described herein such as the compound of Formulae (I) to (XIV). In some embodiments, the inhibitors are able to covalently bind to cysteine. In some other embodiments, the inhibitors inhibit the kinase by bonding to the sulfhydryl group of the cysteine residue at the gatekeeper amino acid position. In some embodiments, a compound provided herein may be a Lrrk-2 kinase inhibitor. In some embodiments, the Lrrk-2 kinase inhibitor is one or more of the compounds set forth in Table Z and/or a compound of Formula (XV) to (XXIX).

A person having ordinary skill in the art would immediately take into account the widely known principles of chemical when considering the description of compounds provided herein. Accordingly, where a group may be substituted by one or more of a number of substituents, such substitutions are selected so as to comply with principles of chemical bonding and to give compounds which are not inherently unstable and/or would be known to one of ordinary skill in the art as likely to be unstable under ambient conditions, such as aqueous, or neutral conditions.

It will be apparent to one skilled in the art that certain compounds of this invention may exist in tautomeric forms, all such tautomeric forms of the compounds being within the scope of the invention.

Unless otherwise stated, structures depicted herein are also meant to include compounds which differ only in the presence of one or more isotopically enriched atoms. For example, compounds having the present structures except for the replacement of a hydrogen by a deuterium or tritium, the replacement of a carbon by  $^{13}\text{C}$ - or  $^{14}\text{C}$ -enriched carbon, or the replacement of an iodine by  $^{125}\text{I}$ , are within the scope of this invention. All isotopic variations of the compounds of the present invention, whether radioactive or not, are encompassed within the scope of the present invention.

The compounds of the present invention also include the salts, hydrates, solvates and prodrug forms. The compounds of the present invention also include the isomers and metabolites of those described in Formula (I)-(XXIX).

Salts include, but are not limited to, sulfate, citrate, acetate, oxalate, chloride, bromide, iodide, nitrate, bisulfate, phosphate, acid phosphate, phosphonic acid, isonicotinate, lactate, salicylate, citrate, tartrate, oleate, tannate, pantothenate, bitartrate, ascorbate, succinate, maleate, gentisinate, fumarate, gluconate, glucuronate, saccharate, formate, benzoate, glutamate, methanesulfonate, ethanesulfonate, benzenesulfonate, p-toluenesulfonate, and pamoate (i.e., 1,1'-methylene-bis-(2-hydroxy-3-naphthoate)) salts. Other salts include, but are not limited to, salts with inorganic bases including alkali metal salts such as sodium salts, and potassium salts; alkaline earth metal salts such as calcium salts, and magnesium salts; aluminum salts; and ammonium salts. Other salts with organic bases include salts with diethylamine, diethanolamine, meglumine, and N,N'-dibenzylethylenediamine.

The neutral forms of the compounds can be regenerated by contacting the salt with a base or acid and isolating the parent compound in the conventional manner. The parent form of the compound differs from the various salt forms in certain physical properties, such as solubility in polar solvents, but otherwise the salts are equivalent to the parent form of the compound for the purposes of the present invention.

Certain compounds of the present invention can exist in unsolvated forms as well as solvated forms, including hydrated forms. In general, the solvated forms are equivalent to unsolvated forms and are encompassed within the scope of the present invention. Certain compounds of the present invention may exist in multiple crystalline or amorphous forms. In general, all physical forms are equivalent for the uses contemplated by the present invention and are intended to be within the scope of the present invention.

Certain compounds of the present invention possess asymmetric carbon atoms (optical centers) or double bonds; the enantiomers, racemates, diastereomers, tautomers, geometric isomers, stereoisometric forms that may be defined, in terms of absolute stereochemistry, as (R)- or (S)- or, as (D)- or (L)- for amino acids, and individual isomers are encompassed within the scope of the present invention. The compounds of the present invention do not include those which are known in art to be too unstable to synthesize and/or isolate. The present invention is meant to include compounds in racemic and optically pure forms. Optically active (R)- and (S)-, or (D)- and (L)-isomers may be prepared using chiral synthons or chiral reagents, or resolved using conventional techniques.

The present invention also provides compounds which are in a prodrug form. Prodrugs of the compounds described herein are those compounds that readily undergo chemical changes under physiological conditions to provide the compounds of the present invention. Additionally, prodrugs can

be converted to the compounds of the present invention by chemical or biochemical methods in an ex vivo environment. For example, prodrugs can be slowly converted to the compounds of the present invention when placed in a transdermal patch reservoir with a suitable enzyme or chemical reagent.

In some embodiments, each substituted group described above for the compounds of the present invention is substituted with at least one substituent group. More specifically, in some embodiments, each substituted alkyl, substituted heteroalkyl, substituted cycloalkyl, substituted heterocycloalkyl, substituted aryl, substituted heteroaryl, substituted or unsubstituted alkylene, substituted or unsubstituted heteroalkylene, substituted or unsubstituted cycloalkylene, substituted or unsubstituted heterocycloalkylene, substituted or unsubstituted arylene or substituted or unsubstituted heteroarylene described above is substituted with at least one substituent group. In other embodiments, at least one or all of these groups are substituted with at least one size-limited substituent group. Alternatively, at least one or all of these groups are substituted with at least one lower substituent group.

In other embodiments of the compounds described above, each substituted or unsubstituted alkyl is a substituted or unsubstituted  $\text{C}_1\text{-C}_{20}$  alkyl, each substituted or unsubstituted alkylene is a substituted or unsubstituted  $\text{C}_1\text{-C}_{20}$  alkylene, each substituted or unsubstituted heteroalkyl is a substituted or unsubstituted 2 to 20 membered heteroalkyl, each substituted or unsubstituted heteroalkylene is a substituted or unsubstituted 2 to 20 membered heteroalkylene, each substituted or unsubstituted cycloalkyl is a substituted or unsubstituted  $\text{C}_3\text{-C}_8$  cycloalkyl, each substituted or unsubstituted cycloalkylene is a substituted or unsubstituted  $\text{C}_3\text{-C}_8$  cycloalkylene, each substituted or unsubstituted heterocycloalkyl is a substituted or unsubstituted 4 to 8 membered heterocycloalkyl, each substituted or unsubstituted heterocycloalkylene is a substituted or unsubstituted 4 to 8 membered heterocycloalkylene, each substituted or unsubstituted aryl is a substituted or unsubstituted  $\text{C}_6$  or  $\text{C}_8$  aryl, each substituted or unsubstituted arylene is a substituted or unsubstituted  $\text{C}_6$  or  $\text{C}_8$  arylene, each substituted or unsubstituted heteroaryl is a substituted or unsubstituted  $\text{C}_5$  or  $\text{C}_6$  heteroaryl, and each substituted or unsubstituted heteroarylene is a substituted or unsubstituted  $\text{C}_5$  or  $\text{C}_6$  heteroarylene.

Alternatively, each substituted or unsubstituted alkyl is a substituted or unsubstituted  $\text{C}_1\text{-C}_8$  alkyl, each substituted or unsubstituted alkylene is a substituted or unsubstituted  $\text{C}_1\text{-C}_8$  alkylene, each substituted or unsubstituted heteroalkyl is a substituted or unsubstituted 2 to 8 membered heteroalkyl, each substituted or unsubstituted heteroalkylene is a substituted or unsubstituted 2 to 8 membered heteroalkylene, each substituted or unsubstituted cycloalkyl is a substituted or unsubstituted  $\text{C}_3\text{-C}_6$  cycloalkyl, each substituted or unsubstituted cycloalkylene is a substituted or unsubstituted  $\text{C}_3\text{-C}_6$  cycloalkylene, each substituted or unsubstituted heterocycloalkyl is a substituted or unsubstituted 3 to 6 membered heterocycloalkyl, and each substituted or unsubstituted heterocycloalkylene is a substituted or unsubstituted 3 to 6 membered heterocycloalkylene.

V. Kinases

In some embodiments, the present invention provides a recombinant kinase comprising a cysteine substitution at a

gatekeeper amino acid position (also referred to as a “cysteine gatekeeper kinase”, a “recombinant kinase of the present invention” or a “recombinant kinase set forth herein”). For example, the recombinant kinase can comprise a sequence having a cysteine substitution at the position corresponding to Thr338 of c-Src, such as the positions shown for SEQ ID NOs:58-77, and sequences having substantial identity thereto. That is, the recombinant kinase can comprise a sequence having at least about 85, 90, 92, 93, 94, 95, 96, 97, 98, or 99% identity to a sequence of any one of SEQ ID NOs:58-77, with a cysteine substitution at the position corresponding to Thr338 of c-Src.

In some embodiments, the recombinant kinase can have a sequence of SEQ ID NO:2 (T338C c-Src), or a sequence having substantial identity thereto. In some embodiments the recombinant kinase can comprise a sequence having at least about 85, 90, 92, 93, 94, 95, 96, 97, 98, or 99% identity to the sequence of SEQ ID NO:2 with a cysteine at the position corresponding to 338 (with reference to the full length sequence of SEQ ID NO:3). In some embodiments, the recombinant kinase comprises less than the full length of SEQ ID NO:2 or 3 (and substantially identical variants thereof), but retains the cysteine substitution at the position corresponding to amino acid 338 of SEQ ID NO:3. In some embodiments, the recombinant kinase comprises at least 8, 10, 12, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 120, 150, 175, 200 or more contiguous amino acids of SEQ ID NO:2 or a substantially identical sequence over that span retaining a cysteine at the amino acid position corresponding to 338 of SEQ ID NO:3.

In some embodiments, the recombinant kinase can have a sequence of any one of SEQ ID NOs:24-45 or a sequence having substantial identity thereto. These kinase sequences have a naturally occurring gatekeeper cysteine, i.e. a cysteine at the position corresponding to amino acid 338 in c-Src (SEQ ID NO:2 shows the T338C c-Src, while SEQ ID NO:4 shows the wild type T338 c-Src sequence). In some embodiments the recombinant kinase can comprise a sequence having at least about 85, 90, 92, 93, 94, 95, 96, 97, 98, or 99% identity to the sequence of any one of SEQ ID NOs:24-45 with a cysteine at the position corresponding to amino acid 338 of c-Src. In some embodiments, the recombinant kinase comprises less than the full length of any one of SEQ ID NOs:24-45 (and substantially identical variants thereof), but retains the cysteine at the position corresponding to amino acid 338 of c-Src. In some embodiments, the recombinant kinase comprises at least 8, 10, 12, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 120, 150, 175, 200 or more contiguous amino acids of any one of SEQ ID NOs:24-45 or a substantially identical sequence over that span retaining a cysteine at the position corresponding to 338 of SEQ ID NO:2.

In some other embodiments, the recombinant kinase has a  $k_{cat}$  activity that is not substantially lower than the  $k_{cat}$  activity of the corresponding wild-type kinase. In some embodiments, the  $k_{cat}$  activity that is not substantially lower than the  $k_{cat}$  activity of the corresponding wild-type kinase. In some embodiments, the recombinant kinase has a  $K_m$  binding affinity for ATP of the recombinant kinase is not substantially lower than the  $K_m$  binding affinity for ATP of the corresponding wild type kinase. In some embodiments, the  $K_m$  binding affinity for ATP of the recombinant kinase is not substantially lower than the  $K_m$  binding affinity for ATP of the correspond-

ing wild type kinase. The activity is considered “not substantially lower” when the activity is not less than 5-fold less, e.g., 4-fold, 3-fold, or 2-fold less than the reference kinase. In some cases, the term “not substantially lower” is determined in terms of percentage, and a not substantially lower activity is at least 50% of the reference kinase, e.g. higher than 50% of the activity of a wild type kinase. In some embodiments, the activity is 60, 70, 75, 80, 85, 90, 95% or higher of the activity of the reference kinase.

The present invention provides methods for evaluating the use of a cysteine gatekeeper kinase.

In some embodiments, the recombinant kinase includes a recombinant kinase is selected from Src (e.g., c-Src (SEQ ID NOs:2-23 or 59) or v-Src (SEQ ID NO: 46-50 or 58); MOK; Sgk494; Yak/Yrk; SRPK1; CDK; DICTY-I; PAK/STE20; or Ctrl/DPYK1 with a cysteine at the gatekeeper position (at the amino acid position corresponding to 338 of the c-Src protein of SEQ ID NO:3). In some embodiments, the recombinant kinase is a recombinant Src and the gatekeeper amino acid position is T338.

In some embodiments, the recombinant kinase has a greater catalytic efficiency than the corresponding wild type kinase. For example, the kinase activity is greater than 100% of the reference kinase (e.g., wild type c-Src of SEQ ID NO:3). In some embodiments, the activity is 1.2-fold, 1.5-fold, 1.8-fold, 2-fold, 2.5-fold, 3-fold, 4-fold, 5-fold, 10-fold or higher than the reference. In some embodiments, the catalytic efficiency is measured as the ratio of  $k_{cat}/K_m$ .

In some embodiments, the recombinant kinase further comprises an additional amino acid substitution corresponding to position V323 of c-Src (see, e.g., SEQ ID NOs:10-23). That is, the recombinant kinase can be a cysteine gatekeeper kinase, i.e., comprising a sequence having substantial identity to any one of SEQ ID NOs:2-77 with a cysteine at the position corresponding to amino acid 338 of SEQ ID NO:3, and additionally include a substitution at the position corresponding to amino acid 323 of SEQ ID NO:3. One of skill will understand that the positions corresponding to those of SEQ ID NO:3 can be ascertained for other kinase sequences.

In some embodiments, the recombinant kinase further comprises an additional amino acid substitution corresponding to the position M314 of c-Src, e.g. to gly (G) or ala (A). For example, the recombinant kinase can be a cysteine gatekeeper kinase, i.e., comprising a sequence having substantial identity to any one of SEQ ID NOs:2-77 with a cysteine at the position corresponding to amino acid 338 of SEQ ID NO:3, and additionally include a substitution at the position corresponding to amino acid 314 of SEQ ID NO:3. One of skill will understand that the positions corresponding to those of SEQ ID NO:3 can be ascertained for other kinase sequences.

In some embodiments, the recombinant kinase includes substitutions at two or all three positions corresponding to positions 338, 314 and 323 of c-Src (SEQ ID NO:3). In some embodiments, the recombinant kinase comprises a sequence having substantial identity to SEQ ID NO:2 with a C at the position corresponding to amino acid 338 of c-Src (the full length sequence of SEQ ID NO:3), and also has a substitution at the position corresponding to amino acid 314 of c-Src. In some embodiments, the recombinant kinase comprises a sequence having substantial identity to SEQ ID NO:2 with a C at the position corresponding to amino acid 338 (of the full

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length sequence of SEQ ID NO:3), and also has a substitution at the position corresponding to amino acid 323 of c-Src. In some embodiments, the recombinant kinase comprises a sequence having substantial identity to SEQ ID NO:2 with a C at the position corresponding to amino acid 338 of c-Src (the full length sequence of SEQ ID NO:3), and also has a substitution at the positions corresponding to amino acids 314 and 323 of c-Src. Again, one of skill will be able to determine the corresponding amino acid positions for kinases with sequences that are not perfectly aligned with c-Src.

In some embodiments, the cysteine gatekeeper kinase has an additional amino acid substitution of alanine (A) or serine (S) at the position corresponding to V323 of c-Src (V323A (c-Src-ES2)) or V323S (c-Src-ES3). In some other embodiments, the recombinant kinase having an additional amino acid substitution at VAL323 has a greater catalytic efficiency of the corresponding recombinant kinase that does not have an additional amino acid substitution at VAL323. In some embodiments, the catalytic efficiency is measured as the ratio of  $k_{cat}/K_m$ .

In some embodiments, the corresponding substitutions can be performed in other kinases. A person having ordinary skill in the art would understand which amino acids correspond to VAL 323 in other kinases.

In some embodiments, the present invention provides methods and compositions for modifying the microenvironment around the cysteine gatekeeper by alteration of one nearby residue (e.g. Val323) in order to impact inhibitor potency. For example, liberating additional space with a V323A mutation resulted in a 5-fold increase in potency for 13, while the V323S mutation had a 12-fold effect. In some embodiments, the present invention provides methods of boosting potency which may allow dosing levels sufficient to substantially minimize off-target effects with MOK kinase (the effects due to MOK inhibition can be taken into account by comparing effects in WT vs. ES expressing cells).

#### A. Forming a Kinase

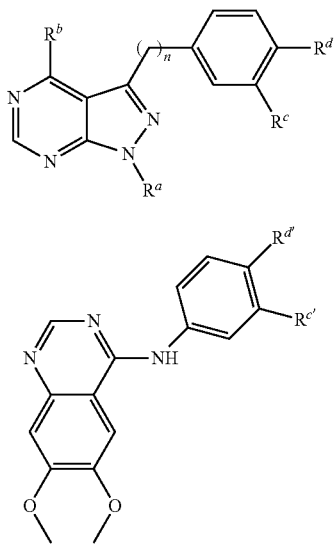
In some other embodiments, the present invention provides a method of forming a recombinant kinase described herein, wherein the method includes transforming a cell with a nucleic acid encoding a recombinant kinase described herein, thereby forming a recombinant kinase described herein. In some embodiments, the recombinant kinase is selected from Src; MOK; Sgk494; Lrrk-2; Yak/Yrk; SRPK1; CDK; DICTY-I; PAK/STE20; or Ctrl/DPYK1 as described herein.

#### B. Structure Activity Relationship Studies—Inhibition of Src

In some embodiment, the present invention provides a series of 3-phenyl-substituted pyrazolopyrimidines with electrophilic groups at positions expected to be in close proximity to the gatekeeper residue and as set forth in Table 1. In some other embodiments, the electrophiles include meta and para substituents of the 3-phenyl ring and vinylsulfonamides as well as acrylamides and chloroacetamides. A meta-substituted vinylsulfonamide, 3 inhibited T338C relative to WT c-Src (>9-fold increase), while a para-substituted version, 5, elicited a ~6-fold improvement (Table 1). Acrylamides (1) and chloroacetamides (6) were also shown to be inhibitors. Under the assay conditions used (10 min preincubation with inhibitor prior to addition of ATP)  $IC_{50}$  values under 5  $\mu$ M for either 2 or 4 for WT or T338C c-Src were not shown.

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TABLE 1



Compound	n	R <sup>a</sup>	R <sup>b</sup>	R <sup>c</sup>	R <sup>d</sup>	WT c-Src IC <sub>50</sub> (nM)	T338C c-Src IC <sub>50</sub> (nM)
1	0	iPr	NH <sub>2</sub>	NHCOCHCH <sub>2</sub>	H	2319	419
2	0	iPr	H	NHCOCHCH <sub>2</sub>	H	>5000	>5000
3	0	iPr	NH <sub>2</sub>	NHSO <sub>2</sub> CHCH <sub>2</sub>	H	1004	111
4	0	iPr	H	NHSO <sub>2</sub> CHCH <sub>2</sub>	H	>5000	>5000
5	0	iPr	NH <sub>2</sub>	H	NHSO <sub>2</sub> CHCH <sub>2</sub>	899	145
6	0	iPr	NH <sub>2</sub>	NHCOCH <sub>2</sub> Cl	H	>5000	817
7	1	iPr	NH <sub>2</sub>	NHCOCHCH <sub>2</sub>	H	>5000	2762
8	1	iPr	NH <sub>2</sub>	H	NHCOCHCH <sub>2</sub>	>5000	>5000
9	1	iPr	NH <sub>2</sub>	NHSO <sub>2</sub> CHCH <sub>2</sub>	H	>5000	150
10	1	iPr	NH <sub>2</sub>	H	NHSO <sub>2</sub> CHCH <sub>2</sub>	3083	1759
11	1	iPr	NH <sub>2</sub>	NHSO <sub>2</sub> CH <sub>2</sub> CH <sub>3</sub>	H	>5000	3497
12	1	iPr	NH <sub>2</sub>	NHCOCH <sub>2</sub> Cl	H	>5000	>5000
13	1	iPr	NH <sub>2</sub>	COCH <sub>2</sub> F	H	>5000	338
14	1	iPr	NH <sub>2</sub>	COCH <sub>3</sub>	H	>5000	4520
15	1	Me	NH <sub>2</sub>	NHSO <sub>2</sub> CHCH <sub>2</sub>	H	>5000	3161
16	1	tBu	NH <sub>2</sub>	NHSO <sub>2</sub> CHCH <sub>2</sub>	H	>5000	618
17	1	Cp	NH <sub>2</sub>	NHSO <sub>2</sub> CHCH <sub>2</sub>	H	>5000	196

Compound	R <sup>c'</sup>	R <sup>d'</sup>	WT c-Src IC <sub>50</sub> (nM)	T338C c-Src IC <sub>50</sub> (nM)
18	NHCOCHCH <sub>2</sub>	H	>5000	1661
19	NHSO <sub>2</sub> CHCH <sub>2</sub>	H	>5000	1004
20	H	NHSO <sub>2</sub> CHCH <sub>2</sub>	2170	560

$IC_{50}$  values for electrophile derivatized pyrazolopyrimidines and 4-anilinoquinazolines against WT c-Src and T338C c-Src. Scaffolds are depicted such that the hinge-binding element is located on the left. Note that for covalent inhibitors  $IC_{50}$  values are time-dependent. In these assays, the inhibitors were preincubated with the Src for ten minutes prior to assay initialization by addition of ATP.

An array of 3-benzyl-substituted pyrazolopyrimidines modified with electrophiles or isosteric and unreactive negative control groups at the meta and para positions were synthesized and screened against WT and T338C c-Src (compounds 7-17, Table 1). The benzyl functionalized compounds inhibited wild type c-Src ( $IC_{50}$  values >5  $\mu$ M). Compound, 9, which is functionalized with a vinylsulfonamide, exhibited an  $IC_{50}$  value of 150 nM. An unreactive control compound 11 resulted in a 23-fold drop in potency. A fluoromethylketone bearing compound, 13, yielded an  $IC_{50}$  value of 338 nM, which was >13-fold more potent than the corresponding ketone, 14.

The present invention also provides methods of determining the activity effects of modifying the N1 position of pyrazolopyrimidines by a structure activity relationship (SAR). In some embodiments, this includes using the pyrazolopyrimi-

dine scaffold with a benzyl-linked m-vinylsulfonamide, see compounds 9, 15-17; Table 1. This analysis revealed that secondary alkyl groups such as isopropyl (9) and cyclopentyl (17) moieties inhibit T338C c-Src. These results indicate that substitution at N1 can be used to modulate potency against T338C c-Src. Accordingly, the present invention provides methods of modulating the potency against kinases, such as c-Src.

The present invention provides methods and compositions that are suitable for use with a variety of kinases, e.g. recombinant, wild type, natural, mutant, and unmutated. In some embodiments, these kinases include c-Src, Src; MOK; Sgk494; Yak/Yrk; SRPK1; CDK; DICTY-I; PAK/STE20; or Ctrl/DPYK1.

In some embodiments, the recombinant kinases described herein include an approximate 15 residue His tag in addition to the sequence for the actual protein, e.g. linker and heptamer for specific TEV protein cutting. In some instances TEV may be cut at residue 248, 249, or 250. It is understood by those in the art that the DNA sequence can be optimized with respect to the code or sequence without affecting the primary protein encoded thereby.

The following sets forth gatekeeper residues. In some embodiments, the gatekeeper residue is cysteine. In some embodiments the kinase is natural, wild type, or recombinant.

As described herein, a Cys gatekeeper is an attractive target for the inhibitory compounds described herein. Representative kinases having a naturally occurring Cys at the gatekeeper position include the entries of Table 2 following. As customary in the art, the terms "GI: number," "GI: No." and the like refer to a unique sequence identifier (i.e., "GenBank Identifier") for a sequence.

TABLE 2

SEQ ID NO:	GI: No.	Species
24	4587987	<i>Arabidopsis thaliana</i>
25	19424095	<i>Arabidopsis thaliana</i>
26	1785621	<i>Arabidopsis thaliana</i>
27	4678270	<i>Arabidopsis thaliana</i>
28	4678272	<i>Arabidopsis thaliana</i>
29	4678273	<i>Arabidopsis thaliana</i>
30	4678277	<i>Arabidopsis thaliana</i>
31	4886274	<i>Arabidopsis thaliana</i>
32	3047095	<i>Arabidopsis thaliana</i>
33	334188021	<i>Arabidopsis thaliana</i>
34	9294588	<i>Arabidopsis thaliana</i>
35	11120792	<i>Arabidopsis thaliana</i>
36	11120796	<i>Arabidopsis thaliana</i>
37	8777331	<i>Arabidopsis thaliana</i>
38	7106391	<i>Mus musculus</i>
39	1705720	<i>Carassius auratus</i>
40	6648996	<i>Capsicum annuum</i>
41	7630151	<i>Leishmania major</i>
42	5139689	<i>Homo sapiens</i>
43	486948	<i>Trichomonas vaginalis</i>
44	254688446	<i>Plasmodium falciparum</i>
45	13509297	<i>Dictyostelium discoideum</i>

## VI. Co-Crystals of Kinase and a Compound

The present invention provides co-crystals of a kinase and a compound, e.g. co-crystal structure of T338C c-Src with a vinylsulfonamide-derivatized pyrazolopyrimidine inhibitor is provided, see Example 34.

In the 9-c-Src-ES1 co-crystal structure, the pyrazolopyrimidine pharmacophore interacts with the backbone amides of Glu339 and Met341 of the hinge region (FIG. 2A). The oxygen atoms of the sulfonamide hydrogen bond directly to the backbone amide of Asp404 and to that of Phe405 via a water molecule (FIG. 2B, C). Additionally, the nitrogen of the sul-

fonamide makes a direct hydrogen bond to the side chain Glu310 (FIGS. 2A, C). In crystal structures of wild type Src, the hydroxyl of the gatekeeper threonine is often directed towards the C4-exocyclic amine of the adenine portion of ATP mimetics.

In the 9 c-Src-ES1 co-crystal structure, the sulfhydryl of Cys338 adopts a distinct rotamer to accommodate the bulky C-3 benzyl group and facilitate a covalent bond (FIG. 2C).

The flexible ethylsulfonamide moiety is situated to allow the covalent linkage with Cys338 (FIG. 2B). The side chain of Met314, a critical component of the hydrophobic spine, is dramatically shifted relative to its position in other c-Src structures (FIGS. 2B, C). Movement of Met314 may prevent a steric clash with the ethylsulfonamide moiety of 9.

## VII. Pharmaceutical Compositions

In some embodiments, the present invention provides a pharmaceutical composition comprising a compound as set forth herein (e.g. a compound of Formula (I)-(XXIX)) and a pharmaceutically acceptable excipient.

"Pharmaceutically acceptable excipient" and "pharmaceutically acceptable carrier" refer to a substance that aids the administration of an active agent to and absorption by a subject and can be included in the compositions of the present invention without causing a significant adverse toxicological effect on the patient. Non-limiting examples of pharmaceutically acceptable excipients include water, NaCl, normal saline solutions, lactated Ringer's, normal sucrose, normal glucose, binders, fillers, disintegrants, lubricants, coatings, sweeteners, flavors and colors, and the like. One of skill in the art will recognize that other pharmaceutical excipients are useful in the present invention.

The compounds and compositions of the present invention can be prepared and administered in a wide variety of oral, parenteral and topical dosage forms. Oral preparations include tablets, pills, powder, dragees, capsules, liquids, lozenges, cachets, gels, syrups, slurries, suspensions, etc., suitable for ingestion by the patient. The compounds of the present invention can also be administered by injection, that is, intravenously, intramuscularly, intracutaneously, subcutaneously, intraduodenally, or intraperitoneally. Also, the compounds described herein can be administered by inhalation, for example, intranasally. Additionally, the compounds and compositions of the present invention can be administered transdermally. The GR modulators of this invention can also be administered by intraocular, intravaginal, and intrarectal routes including suppositories, insufflation, powders and aerosol formulations (for examples of steroid inhalants, see Rohatagi, *J. Clin. Pharmacol.* 35:1187-1193, 1995; Tjwa, *Ann. Allergy Asthma Immunol.* 75:107-111, 1995). Accordingly, the present invention also provides pharmaceutical compositions including a pharmaceutically acceptable carrier or excipient and either a compound of Formula I, or a pharmaceutically acceptable salt of a compound of Formula I.

For preparing pharmaceutical compositions from the compounds of the present invention, pharmaceutically acceptable carriers can be either solid or liquid. Solid form preparations include powders, tablets, pills, capsules, cachets, suppositories, and dispersible granules. A solid carrier can be one or more substances, which may also act as diluents, flavoring agents, binders, preservatives, tablet disintegrating agents, or an encapsulating material. Details on techniques for formulation and administration are well described in the scientific and patent literature, see, e.g., the latest edition of Remington's Pharmaceutical Sciences, Maack Publishing Co, Easton Pa. ("Remington's").

In powders, the carrier is a finely divided solid, which is in a mixture with the finely divided active component. In tablets,

the active component is mixed with the carrier having the necessary binding properties in suitable proportions and compacted in the shape and size desired. The powders and tablets preferably contain from 5% or 10% to 70% of the active compound.

Suitable solid excipients include, but are not limited to, magnesium carbonate; magnesium stearate; talc; pectin; dextrin; starch; tragacanth; a low melting wax; cocoa butter; carbohydrates; sugars including, but not limited to, lactose, sucrose, mannitol, or sorbitol, starch from corn, wheat, rice, potato, or other plants; cellulose such as methyl cellulose, hydroxypropylmethyl-cellulose, or sodium carboxymethyl-cellulose; and gums including arabic and tragacanth; as well as proteins including, but not limited to, gelatin and collagen. If desired, disintegrating or solubilizing agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, alginic acid, or a salt thereof, such as sodium alginate.

Dragee cores are provided with suitable coatings such as concentrated sugar solutions, which may also contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for product identification or to characterize the quantity of active compound (i.e., dosage). Pharmaceutical preparations of the invention can also be used orally using, for example, push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a coating such as glycerol or sorbitol. Push-fit capsules can contain GR modulator mixed with a filler or binders such as lactose or starches, lubricants such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the GR modulator compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycol with or without stabilizers.

For preparing suppositories, a low melting wax, such as a mixture of fatty acid glycerides or cocoa butter, is first melted and the active component is dispersed homogeneously therein, as by stirring. The molten homogeneous mixture is then poured into convenient sized molds, allowed to cool, and thereby to solidify.

Liquid form preparations include solutions, suspensions, and emulsions, for example, water or water/propylene glycol solutions. For parenteral injection, liquid preparations can be formulated in solution in aqueous polyethylene glycol solution.

Aqueous solutions suitable for oral use can be prepared by dissolving the active component in water and adding suitable colorants, flavors, stabilizers, and thickening agents as desired. Aqueous suspensions suitable for oral use can be made by dispersing the finely divided active component in water with viscous material, such as natural or synthetic gums, resins, methylcellulose, sodium carboxymethylcellulose, hydroxypropylmethylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia, and dispersing or wetting agents such as a naturally occurring phosphatide (e.g., lecithin), a condensation product of an alkylene oxide with a fatty acid (e.g., polyoxyethylene stearate), a condensation product of ethylene oxide with a long chain aliphatic alcohol (e.g., heptadecaethylene oxycetanol), a condensation product of ethylene oxide with a partial ester derived from a fatty acid and a hexitol (e.g., polyoxyethylene sorbitol mono-oleate), or a condensation product of ethylene oxide with a partial ester derived from fatty acid and a hexitol anhydride (e.g., polyoxyethylene sorbitan mono-oleate). The aqueous suspension can also contain one or more preservatives such as ethyl or n-propyl p-hydroxybenzoate, one or more coloring agents, one or more flavoring agents and one or

more sweetening agents, such as sucrose, aspartame or saccharin. Formulations can be adjusted for osmolarity.

Also included are solid form preparations, which are intended to be converted, shortly before use, to liquid form preparations for oral administration. Such liquid forms include solutions, suspensions, and emulsions. These preparations may contain, in addition to the active component, colorants, flavors, stabilizers, buffers, artificial and natural sweeteners, dispersants, thickeners, solubilizing agents, and the like.

Oil suspensions can be formulated by suspending a GR modulator in a vegetable oil, such as arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin; or a mixture of these. The oil suspensions can contain a thickening agent, such as beeswax, hard paraffin or cetyl alcohol. Sweetening agents can be added to provide a palatable oral preparation, such as glycerol, sorbitol or sucrose. These formulations can be preserved by the addition of an antioxidant such as ascorbic acid. As an example of an injectable oil vehicle, see Minto, *J. Pharmacol. Exp. Ther.* 281:93-102, 1997. The pharmaceutical formulations of the invention can also be in the form of oil-in-water emulsions. The oily phase can be a vegetable oil or a mineral oil, described above, or a mixture of these. Suitable emulsifying agents include naturally-occurring gums, such as gum acacia and gum tragacanth, naturally occurring phosphatides, such as soybean lecithin, esters or partial esters derived from fatty acids and hexitol anhydrides, such as sorbitan mono-oleate, and condensation products of these partial esters with ethylene oxide, such as polyoxyethylene sorbitan mono-oleate. The emulsion can also contain sweetening agents and flavoring agents, as in the formulation of syrups and elixirs. Such formulations can also contain a demulcent, a preservative, or a coloring agent.

#### VIII. Administration

The compositions of the present invention can be delivered by transdermally, by a topical route, formulated as applicator sticks, solutions, suspensions, emulsions, gels, creams, ointments, pastes, jellies, paints, powders, and aerosols.

The compositions of the present invention can also be delivered as microspheres for slow release in the body. For example, microspheres can be administered via intradermal injection of drug-containing microspheres, which slowly release subcutaneously (see Rao, *J. Biomater Sci. Polym. Ed.* 7:623-645, 1995; as biodegradable and injectable gel formulations (see, e.g., Gao *Pharm. Res.* 12:857-863, 1995); or, as microspheres for oral administration (see, e.g., Eyles, *J. Pharm. Pharmacol.* 49:669-674, 1997). Both transdermal and intradermal routes afford constant delivery for weeks or months.

The pharmaceutical compositions of the present invention can be provided as a salt and can be formed with many acids, including but not limited to hydrochloric, sulfuric, acetic, lactic, tartaric, malic, succinic, etc. Salts tend to be more soluble in aqueous or other protonic solvents that are the corresponding free base forms. In other cases, the preparation may be a lyophilized powder in 1 mM-50 mM histidine, 0.1%-2% sucrose, 2%-7% mannitol at a pH range of 4.5 to 5.5, that is combined with buffer prior to use.

In another embodiment, the compositions of the present invention are useful for parenteral administration, such as intravenous (IV) administration or administration into a body cavity or lumen of an organ. The formulations for administration will commonly comprise a solution of the compositions of the present invention dissolved in a pharmaceutically acceptable carrier. Among the acceptable vehicles and solvents that can be employed are water and Ringer's solution,

an isotonic sodium chloride. In addition, sterile fixed oils can conventionally be employed as a solvent or suspending medium. For this purpose any bland fixed oil can be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid can likewise be used in the preparation of injectables. These solutions are sterile and generally free of undesirable matter. These formulations may be sterilized by conventional, well known sterilization techniques. The formulations may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjusting agents, e.g., sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate and the like. The concentration of the compositions of the present invention in these formulations can vary widely, and will be selected primarily based on fluid volumes, viscosities, body weight, and the like, in accordance with the particular mode of administration selected and the patient's needs. For IV administration, the formulation can be a sterile injectable preparation, such as a sterile injectable aqueous or oleaginous suspension. This suspension can be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation can also be a sterile injectable solution or suspension in a nontoxic parenterally-acceptable diluent or solvent, such as a solution of 1,3-butanediol.

In another embodiment, the formulations of the compositions of the present invention can be delivered by the use of liposomes which fuse with the cellular membrane or are endocytosed, i.e., by employing ligands attached to the liposome, or attached directly to the oligonucleotide, that bind to surface membrane protein receptors of the cell resulting in endocytosis. By using liposomes, particularly where the liposome surface carries ligands specific for target cells, or are otherwise preferentially directed to a specific organ, one can focus the delivery of the compositions of the present invention into the target cells in vivo. (See, e.g., Al-Muhammed, *J. Microencapsul.* 13:293-306, 1996; Chonn, *Curr. Opin. Biotechnol.* 6:698-708, 1995; Ostro, *Am. J. Hosp. Pharm.* 46:1576-1587, 1989).

The pharmaceutical preparation is preferably in unit dosage form. In such form the preparation is subdivided into unit doses containing appropriate quantities of the active component. The unit dosage form can be a packaged preparation, the package containing discrete quantities of preparation, such as packaged tablets, capsules, and powders in vials or ampoules. Also, the unit dosage form can be a capsule, tablet, cachet, or lozenge itself, or it can be the appropriate number of any of these in packaged form.

The quantity of active component in a unit dose preparation may be varied or adjusted from 0.1 mg to 10000 mg, more typically 1.0 mg to 1000 mg, most typically 10 mg to 500 mg, according to the particular application and the potency of the active component. The composition can, if desired, also contain other compatible therapeutic agents.

The compounds described herein can be used in combination with one another, with other active agents known to be useful in modulating a protein kinase, or with adjunctive agents that may not be effective alone, but may contribute to the efficacy of the active agent.

In some embodiments, co-administration includes administering one active agent within 0.5, 1, 2, 4, 6, 8, 10, 12, 16, 20, or 24 hours of a second active agent. Co-administration includes administering two active agents simultaneously, approximately simultaneously (e.g., within about 1, 5, 10, 15, 20, or 30 minutes of each other), or sequentially in any order. In some embodiments, co-administration can be accom-

plished by co-formulation, i.e., preparing a single pharmaceutical composition including both active agents. In other embodiments, the active agents can be formulated separately. In another embodiment, the active and/or adjunctive agents may be linked or conjugated to one another.

#### IX. Nucleic Acids

In some embodiments, the present invention provides an isolated nucleic acid comprising a nucleic acid sequence encoding a recombinant kinase provided herein (i.e. a recombinant kinase comprising a cysteine substitution at a gatekeeper amino acid position). This is also referred to herein as a "nucleic acid of the present invention." Thus, provided herein are nucleic acids that encode the cysteine gatekeeper kinases described herein, e.g., recombinant kinases having a cysteine in the position corresponding to amino acid 338 of c-Src (SEQ ID NO:3).

In some embodiments, the nucleic acid sequence encodes a sequence or an enzymatically functional fragment thereof, set forth in SEQ ID NOs 2-77. The enzymatically functional fragment may be 50, 100, 150, or 200 bases in length. In some embodiments, the nucleic acid encodes a polypeptide having substantial identity to any one of SEQ ID NOs:2-77 wherein the polypeptide has a cysteine at the position corresponding to amino acid 338 of c-Src. One of skill will understand that a number of nucleic acid sequences will encode the same polypeptide, due to the degeneracy of the nucleic acid code. In some embodiments, the nucleic acid encodes a polypeptide encoding any one of SEQ ID NOs:2-77, wherein the polypeptide has a cysteine at the position corresponding to 338 of c-Src, or a sequence having at least 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identity thereto. In some embodiments, the polypeptide is shorter than the full length of any one of SEQ ID NOs:2-77, but retains enzymatic (kinase) activity. In some embodiments, the polypeptide is at least 25, 30, 40, 50, 75, 80, 100, 120, 150, 200 or more amino acids in length, and has substantial identity over the corresponding length of the selected sequence (selected from the sequences consisting of SEQ ID NOs:2-77, having a C at the position corresponding to 338 of c-Src). For the non-identical amino acids, one of skill will understand that conservative amino acid substitutions can be included.

In some embodiments, the nucleic acid encodes a polypeptide having substantial identity to any one of SEQ ID NOs:2-77 wherein the polypeptide has a cysteine at the position corresponding to amino acid 338 of c-Src, and an additional amino acid substitution at the position corresponding to amino acid 323 of c-Src and/or the position corresponding to amino acid 314 of c-Src. One of skill will understand that a number of nucleic acid sequences will encode the same polypeptide, due to the degeneracy of the nucleic acid code. In some embodiments, the nucleic acid encodes a polypeptide encoding any one of SEQ ID NOs:2-77, wherein the polypeptide has a cysteine at the position corresponding to 338 of c-Src, and optionally one or both of the substitutions at positions corresponding to amino acids 314 or 323 of c-Src, or a sequence having at least 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identity thereto. In some embodiments, the polypeptide is shorter than the full length of any one of SEQ ID NOs:2-77, but retains enzymatic (kinase) activity. In some embodiments, the polypeptide is at least 25, 30, 40, 50, 75, 80, 100, 120, 150, 200 or more amino acids in length, and has substantial identity over the corresponding length of the selected sequence (selected from the sequences consisting of SEQ ID NOs:2-77, having a C at the position corresponding to 338 of c-Src, and optionally one or both of the substitutions at positions corresponding to amino acids 314 or 323 of c-Src).

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In some other embodiments, the present invention provides an expression cassette comprising a nucleic acid of the present invention. In yet other embodiments, the expression cassette is a recombinant viral vector. In some other embodiments, the expression cassette of is inside of a host cell. In other embodiments, the expression cassette is selected from mammalian, non-mammalian, mouse, rat, or human. In some embodiments, the recombinant kinase is inside a cell. In some other embodiments, the cell is selected from mammalian, non-mammalian, mouse, rat, or human. Thus, in some embodiments, a transgenic mouse or rat is provided, wherein

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the transgenic mouse or rat expresses a recombinant kinase comprising a cysteine substitution at a gatekeeper amino acid position as described above. Methods of producing a transgenic mouse or rat that expresses recombinant proteins and enzymes are well-known in the art. A detailed description for such procedures may be found elsewhere, for example at U.S. Pat. No. 4,736,866, the contents of which are incorporated by reference in their entirety for all purposes.

A. Descriptions of SEQ ID NOs (1-51) Follows.

The following sets forth SEQ ID NOs: 1-51 that are suitable for use with the compositions, methods, and kits herein:

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 SEQ ID NO: Description
 

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1	DNA construct for T338C c-src (251-533)
2	Protein encoded by SEQ ID NO: 1
3	<i>Gallus gallus</i> proto-oncogene (c = -Src)
4	c-Src (251-533)
5	c-Src (251-533) with GHM at N-terminal
6	[T338X]c-Src (251-533)
7	GHM-[T338X]c-Src (251-533) (GHM at N-terminal)
8	[T338C]c-Src (251-533) (c-Src "ES1")
9	GHM-[T338C]c-Src (251-533) (GHM at N-terminal) (c-Src "ES1")
10	[T338X, V323X]c-Src (251-533)
11	GHM-[T338X, V323X]c-Src (251-533) (GHM at N-terminal)
12	[T338C, V323X]c-Src (251-533)
13	GHM-[T338C, V323X]c-Src (251-533) (GHM at N-terminal)
14	[T338C, V323A]c-Src (251-533) (c-Src "ES2")
15	GHM-[T338C, V323A]c-Src (251-533) (GHM at N-terminal) (c-Src "ES2")
16	[T338C, V323S]c-Src (251-533) (c-Src "ES3")
17	GHM-[T338C, V323S]c-Src (251-533) (GHM at N-terminal) (c-Src "ES3")
18	[T338C, V323D]c-Src (251-533) (c-Src "ES4")
19	GHM-[T338C, V323D]c-Src (251-533) (GHM at N-terminal) (c-Src "ES4")
20	[T338C, V323E]c-Src (251-533) (c-Src "ES5")
21	GHM-[T338C, V323E]c-Src (251-533) (GHM at N-terminal) (c-Src "ES5")
22	[T338C, V323H]c-Src (251-533) (c-Src "ES6")
23	GHM-[T338C, V323H]c-Src (251-533) (GHM at N-terminal) (c-Src "ES6")
24	Kinases with gatekeeper Cys ( <i>Arabidopsis thaliana</i> ) 4587987
25	Kinases with gatekeeper Cys ( <i>Arabidopsis thaliana</i> ) 19424095
26	Kinases with gatekeeper Cys ( <i>Arabidopsis thaliana</i> ) 1785621
27	Kinases with gatekeeper Cys ( <i>Arabidopsis thaliana</i> ) 4678270
28	Kinases with gatekeeper Cys ( <i>Arabidopsis thaliana</i> ) 4678272
29	Kinases with gatekeeper Cys ( <i>Arabidopsis thaliana</i> ) 4678273
30	Kinases with gatekeeper Cys ( <i>Arabidopsis thaliana</i> ) 4678277
31	Kinases with gatekeeper Cys ( <i>Arabidopsis thaliana</i> ) 4886274
32	Kinases with gatekeeper Cys ( <i>Arabidopsis thaliana</i> ) 3047095
33	Kinases with gatekeeper Cys ( <i>Arabidopsis thaliana</i> ) 334188021/15238494
34	Kinases with gatekeeper Cys ( <i>Arabidopsis thaliana</i> ) 9294588
35	Kinases with gatekeeper Cys ( <i>Arabidopsis thaliana</i> ) 11120792
36	Kinases with gatekeeper Cys ( <i>Arabidopsis thaliana</i> ) 11120796
37	Kinases with gatekeeper Cys ( <i>Arabidopsis thaliana</i> ) 8777331
38	Kinases with gatekeeper Cys ( <i>Mus musculus</i> ) 7106391
39	Kinases with gatekeeper Cys ( <i>Carassius auratus</i> ) 1705720
40	Kinases with gatekeeper Cys ( <i>Capsicum annuum</i> ) 6648996
41	Kinases with gatekeeper Cys ( <i>Leishmania major</i> ) 7630151
42	Kinases with gatekeeper Cys ( <i>Homo sapiens</i> ) 5139689
43	Kinases with gatekeeper Cys ( <i>Trichomonas vaginalis</i> ) 486948
44	Kinases with gatekeeper Cys ( <i>Plasmodium falciparum</i> ) 254688446/3845109
45	Kinases with gatekeeper Cys ( <i>Dictyostelium discoideum</i> ) 13509297
46	v-Src (Rous sarcoma virus)
47	[I338X]v-Src
48	[I338C]v-Src
49	[I338T]v-Src
50	[I338G]v-Src
51	Artificial sequence (substrate for methods of testing)

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An exemplary DNA construct useful for the methods described herein was synthesized (SEQ ID NO: 1). This construct encodes the expressed protein set forth in SEQ ID NO:2. The expressed protein includes a His<sub>6</sub> (SEQ ID NO:78) tag sequence at the N-terminal, useful for purification of recombinantly expressed protein as known in the art. The expressed protein further includes a spacer sequence (i.e., DYDIPTT, (SEQ ID NO:79), SEQ ID NO:2 residues 7-13) and a tobacco etch viral (TEV) protease site (i.e., ENLYFQG, (SEQ ID NO: 80), SEQ ID NO:2, residues 14-20) as known in

the art. An additional spacer (e.g., SEQ ID NO:2, residues 21-22) may be present in expressed proteins, which spacers residues may occupy the N-terminal position(s) of the expressed protein after protease cleavage (e.g., TEV protease cleavage). Thus, it is understood that reference to "c-Src (251-533)" and variants thereof herein contemplates expressed proteins having one or more amino acids at the N-terminal which may result from the process of recombinant protein production. For example, after the action of the TEV protease on the protein of SEQ ID NO:2, the expressed c-Src

(251-533) protein may include the N-terminal tripeptide "GHM." It is understood that absent indication otherwise, the numbering of c-Src proteins and variants as discussed herein follows the numbering of the full c-Src protein (SEQ ID NO:3). For example, full length c-Src (SEQ ID NO:3) contains 533 residues. Accordingly, residues 23-305 of SEQ ID NO:2 correspond to residues 251-533 of SEQ ID NO:3. c-Src (251-533) is expressly set forth in SEQ ID NO:4. A recombinantly expressed and processed protein of c-Src (251-533), as described above, having the N-terminal tripeptide "GHM" is set forth in SEQ ID NO:5.

In some embodiments, a c-Src variant is provided wherein the residue at the position equivalent to Thr<sup>338</sup> of c-Src (SEQ ID NO:3) is substituted with another amino acid. In some embodiments, the substituted amino acid is a naturally occurring amino acid, as known in the art. Exemplary recombinantly expressed proteins having this substitution are set forth in SEQ ID NO:6 and SEQ ID NO:7, wherein SEQ ID NO:7 further includes the N-terminal tripeptide "GHM" as described above. Similarly, in some embodiments, a v-Src variant is provided wherein the residue at the position equivalent to Thr<sup>338</sup> of v-Src (SEQ ID NO:46) is substituted with another amino acid. An exemplary recombinantly expressed protein having this substitution is set forth in SEQ ID NO:47. Specific exemplary recombinantly expressed proteins having a C, T or G substitution at position 338 of v-Src (i.e., [I338C] v-Src, [I338T] v-Src, [I338G] v-Src), are set forth in SEQ ID NO: 48, SEQ ID NO:49 and SEQ ID NO:50, respectively.

In some embodiments, a protein kinase is provided having a Thr to Cys substitution at the position corresponding to residue 338 of c-Src (i.e., T338C substitution). The protein may be a fragment of full length c-Src. Recombinantly expressed protein variants of c-Src (251-533) having a T338C substitution (i.e., [T338C]c-Src(251-533)) are set forth in SEQ ID NO:8 and SEQ ID NO:9. It is understood that within the context of protein descriptive names, bracketed (i.e., "[ ]") entries denote substitution(s), and that parenthetical entries after the protein name denote the corresponding residues of the fragment. For example, "[T338C]c-Src(251-533)" refers to the fragment of c-Src from residue 251 to residue 533, additionally having a Thr to Cys substitution at position 338 (c-Src numbering). These proteins are also known as "c-Src ES1" proteins. It is further understood that, as customary in the art, the term "XNNNY" refers to substitution of residue "X" at position "NNN" with residue "Y."

In some embodiments, a plurality of substitutions of c-Src, or fragment thereof, are provided. For example, in some embodiments, a protein having double substitutions at residues T<sup>338</sup> and V<sup>323</sup> of c-Src is provided. In some embodiments, a protein having double substitutions at residues T<sup>338</sup> and V<sup>323</sup> of a fragment of c-Src (e.g., c-Src(251-533)) is provided. See SEQ ID NO:10. In some embodiments, the fragment of c-Src includes an N-terminal oligopeptide sequence resulting from processing of the recombinant protein as described above. See SEQ ID NO:11.

In some embodiments, there is provided a T338C substitution of c-Src, or fragment thereof (e.g., c-Src(251-533), in combination with a substitution at position 323. See SEQ ID NO:12. In some embodiments, such doubly substituted fragment of c-Src includes an N-terminal oligopeptide sequence resulting from processing of the recombinant protein as described above. See SEQ ID NO:13.

In some embodiments, a T338C substitution of c-Src, or fragment thereof (e.g., c-Src(251-533), in combination with a specific substitution at position 323 is provided. For example, [T<sup>338</sup>C, V<sup>323</sup>A]c-Src(251-533) is set forth in SEQ ID NO:14, and the corresponding protein having an N-terminal oli-

gopeptide sequence resulting from processing of the recombinant protein is set forth in SEQ ID NO:15. These doubly substituted proteins are also known as the so-called c-Src "ES2" variant.

Further exemplary of this embodiment, there is provided [T<sup>338</sup>C, V<sup>323</sup>S]c-Src(251-533) (SEQ ID NO:16), and the corresponding protein having an N-terminal oligopeptide sequence resulting from processing of the recombinant protein (SEQ ID NO:17). These doubly substituted proteins are also known as the so-called c-Src "ES3" variant.

Further exemplary of this embodiment, there is provided [T<sup>338</sup>C, V<sup>323</sup>D]c-Src(251-533) (SEQ ID NO:18), and the corresponding protein having an N-terminal oligopeptide sequence resulting from processing of the recombinant protein (SEQ ID NO:19). These doubly substituted proteins are also known as the so-called c-Src "ES4" variant.

Further exemplary of this embodiment, there is provided [T<sup>338</sup>C, V<sup>323</sup>E]c-Src(251-533) (SEQ ID NO:20), and the corresponding protein having an N-terminal oligopeptide sequence resulting from processing of the recombinant protein (SEQ ID NO:21). These doubly substituted proteins are also known as the so-called c-Src "ES5" variant.

Further exemplary of this embodiment, there is provided [T<sup>338</sup>C, V<sup>323</sup>H]c-Src(251-533) (SEQ ID NO:22), and the corresponding protein having an N-terminal oligopeptide sequence resulting from processing of the recombinant protein (SEQ ID NO:23). These doubly substituted proteins are also known as the so-called c-Src "ES6" variant.

## X. Methods

### A. General

In some embodiments, the present invention provides methods of determining the role of a kinase in a cell. In certain embodiments, the methods include determining the dependence of transformed cells on aberrant oncogenic signaling by the EGFR kinase. In other embodiments, the determining includes assaying inhibitor-induced conformational changes of kinases. In other embodiments, the methods include elucidating the mechanisms of inhibitor-induced Akt hyperphosphorylation. In some embodiments, the methods include transactivation of RAF dimmers.

In some other embodiments, the present invention provides a chemical genetic approach based on engineered shape complementarity between the kinase active site and a small molecule inhibitor, which allows systematic discovery of an inhibitor for a particular kinase. In some embodiments, a conserved hydrophobic residue in the kinase active site known as the "gatekeeper" is mutated to a small residue such as glycine or alanine to generate a uniquely targetable mutant kinase termed an analog-sensitive (AS) allele.

In certain other embodiments, the present invention provides methods of making engineered kinase which can be targeted with sterically bulky analogs of natural kinase inhibitors, which are capable of occupying the enlarged engineered kinase pocket (FIG. 1). In some embodiments, the methods include wild type kinases which may be resistant to inhibition by the bulky analog as the result of a steric clash with naturally occurring gatekeeper residues (e.g. Met, Leu, Phe, Thr, Gln and others). In yet other instances, the wild type kinases may not be resistant to inhibition by the bulky analog as the result of a steric clash with naturally occurring gatekeeper residues (e.g. Met, Leu, Phe, Thr, Gln and others).

## B. Structure Activity Relationship (SAR) Analysis

In order to determine the effects of modifying the group at this position, a structure activity relationship (SAR) analysis was performed on the pyrazolopyrimidine scaffold with a benzyl-linked m-vinylsulfonamide (compounds 9, 15-17; Table 2). This analysis revealed that secondary alkyl groups such as isopropyl (9) and cyclopentyl (17) moieties elicited optimal activity against T338C c-Src. Relative to isopropyl substitution, tert-butyl (16) and methyl (15) derivatization resulted in 4- and 21-fold drops in potency, respectively.

Collectively, these results indicate that substitution at N1 can be used to modulate potency against T338C c-Src. IN some instances, Michael acceptor-derivatized 4-anilinoquinazolines were synthesized and evaluated as inhibitors (compounds 18-20; Table 2).

In some embodiments, the ES kinase alleles should be useful for a host of other applications. For example, fluorescently labeled versions of the inhibitors could be used to quantitatively probe the occupancy of kinase active sites to determine the percent activity required for signaling events. In some other embodiments, the present invention provides a method for determining the properties of pseudokinases, for which there is no good readout of active site occupancy. In certain embodiments, the present invention sets forth the use of irreversible inhibitors and allows for the validation of target specificity.

In some embodiments, the present invention provides methods of evaluating the reversibility of inhibition of a kinase as set forth herein. In some embodiments, an electrophilic inhibitors covalently interact with the cysteine gatekeeper. In one instance, two compounds, 9 and 13, were assayed accordingly. Both compounds inhibited T338C c-Src in a time-dependent manner (Table 3).

In addition, when T338C c-Src was treated with either inhibitor and purified by gel filtration and the inhibitory activity against the kinase was retained. See FIG. 5. In contrast, in the case of WT c-Src, inhibitory activity was lost after gel filtration. Importantly, inhibition by PP1, a reversible Src inhibitor, was abrogated in the cases of both WT and T338C c-Src following gel filtration (FIG. 5). Full protein mass spectrometry suggested specific labeling of T338C relative to WT c-Src for 9 (FIG. 2). However, under similar conditions, an adduct formation with 13 was not observed, possibly due to a reversible covalent interaction. The results suggest that covalent binding of the electrophilic inhibitors depend on the presence of a cysteine gatekeeper, i.e. T338C c-Src as electrophile-sensitive c-Src1 (c-Src-ES1).

TABLE 3

Compounds 9 and 13 exhibit time-dependent inhibition against T338C c-Src. Compounds were preincubated with the enzyme prior to initializing the reaction with ATP.			
Compound	Preincubation Time (min)		
	2	20	40
	T338C c-Src IC <sub>50</sub> (nM)		
9	981	309	138
13	1281	318	136

Table 3 shows compounds 9 and 13 exhibit time-dependent inhibition against T338C c-Src. Compounds were preincubated with the enzyme prior to initializing the reaction with ATP.

## XI. Methods of Inhibiting a Kinase

In some other embodiments, the present invention provides a method of imparting to a kinase the capability of being inhibited by a heterocyclic compound, comprising replacing a gatekeeper amino acid residue within an ATP binding site of a kinase with a cysteine residue thereby forming a cysteine substituted kinase.

In some embodiments, the present invention provides a method of inhibiting a recombinant kinase as set forth herein, comprising contacting the recombinant kinase with an effective amount of an inhibitor, thereby inhibiting the recombinant kinase. In some embodiments, the inhibitor is capable of forming a covalent bond to the cysteine at the gatekeeper amino acid position of the recombinant kinase. In some embodiments, the inhibitor is a compound as set forth herein, e.g. a compound of formulas I-XXIX. In some other embodiments, the method further comprises determining a level of inhibition for the recombinant kinase. In some embodiments, the determining of said level of inhibition for the recombinant kinase comprises: determining an amount of enzymatic activity of the recombinant kinase in the presence of the inhibitor; determining an amount of enzymatic activity of the recombinant kinase in the absence of the inhibitor; and comparing the amount of enzymatic activity of the recombinant kinase in the presence of the inhibitor with the amount of enzymatic activity of the recombinant kinase in the absence of the inhibitor, thereby determining a level of inhibition for the recombinant kinase. In some embodiments, the enzymatic activity is selected from phosphorylation of a non-specific protein target, phosphorylation of a specific protein target, consumption of ATP, or cell growth.

In other embodiments, the present invention provides a method as set forth herein wherein the recombinant kinase is in a cell. In some embodiments, the methods set forth herein further comprise determining a function of the recombinant kinase in the cell, by: determining an amount of enzymatic activity of the recombinant kinase in the presence of the inhibitor in the cell; determining an amount of enzymatic activity of the recombinant kinase in the absence of the inhibitor in the cell; and comparing the amount of enzymatic activity of the recombinant kinase in the presence of the inhibitor with the amount of enzymatic activity of the recombinant kinase in the absence of the inhibitor, thereby determining a function of the recombinant kinase in the cell. In some embodiments, the enzymatic activity is selected from phosphorylation of a specific protein target.

In other embodiments, the methods as set forth herein include a recombinant kinase is selected from Src; MOK; Sgk494; Lrrk-2; Yak/Yrk; SRPK1; CDK; DICTY-I; PAK/STE20; or Ctrl/DPYK1. In some embodiments, the recombinant kinase is Src.

In some embodiments, the present invention provides a method of inhibiting a Lrrk-2 kinase, comprising contacting the Lrrk-2 kinase with an effective amount of a Lrrk-2 inhibitor, thereby inhibiting the recombinant Lrrk-2 kinase. A Lrrk-2 inhibitor is compound of Formula (XV) to (XXIX) including embodiments thereof.

## XII. Methods of Treating

In some embodiments, the present invention provides a method of treating a kinase-associated disease or condition, in a patient in need thereof. The method includes administering to the patient a therapeutically effective amount of a compound provided herein, thereby treating a kinase-associated disease or condition. In some embodiments, the compound is a kinase inhibitor capable of forming a covalent bond to the cysteine at the gatekeeper amino acid position of the recombinant kinase. In some embodiments, the compound is a compound as set forth herein, e.g. a compound of formulas (I)-(XXIX) including embodiments thereof. In certain embodiments, the kinase-associated disease or condition is selective from cancer, immunological disorders, neurological disorders, neurodegenerative disorders, infections, metabolic diseases, Leishmania major, zoonotic cutaneous leishmaniasis, Plasmodium falciparum, malaria, Trichomonas vaginalis, and trichomiasis. In certain other embodiments, the cancer is selected from neoplasm or malignant tumors found in mammals; leukemia; carcinomas and sarcomas; cancer of the brain, breast, cervix, colon, head and neck, liver, kidney, lung, non-small cell lung, ovary, testicle, stomach, uterus; melanoma; mesothelioma; Medulloblastoma; Hodgkin's Disease, Non-Hodgkin's Lymphoma; multiple myeloma; neuroblastoma; rhabdomyosarcoma; primary thrombocytosis; primary macroglobulinemia; primary brain tumors; malignant pancreatic insulinoma; malignant carcinoid; urinary bladder cancer; premalignant skin lesions; lymphomas; thyroid cancer; neuroblastoma; esophageal cancer; genitourinary tract cancer; malignant hypercalcemia; endometrial cancer; adrenal cortical cancer; neoplasms of the endocrine and exocrine pancreas; or prostate cancer. In yet other embodiments, the disease or condition is a neurodegenerative disease selective from Parkinson's disease.

In some other embodiments, the present invention also provides a method of treating a Lrrk-2-associated disease or condition, in a patient in need thereof, said method compris-

ing administering to said patient a therapeutically effective amount of a Lrrk-2 inhibitor, thereby treating a Lrrk-2-associated disease or condition. In some embodiments, the disease or condition is a neurodegenerative disease selected from Parkinson's Disease.

In some embodiments, the methods further include the step of allowing the cell to express the recombinant kinase.

## XIII. Tables Relevant to the Methods and Assays Herein

TABLE 4

Relative $k_{cat}/K_m$ for a series of c-Src variants	
c-Src Variant	rel. $k_{cat}/K_m$
ES1	1.00 $\pm$ 0.09
ES2	0.39 $\pm$ 0.03
ES3	0.21 $\pm$ 0.04
ES4	N.D.
ES5	N.D.
ES6	N.D.

Table 5a-5c show relative catalytic efficiency for T338C c-Src with second-site mutations (ES1=T338C; ES2=T338C/V323A; ES3=T338C/V323S, ES4=T338C/V323D; ES5=T338C/V323E; ES6=T338C/V323H). Data were fitted to the Michaelis-Menten equation and standard errors of the fits are reported. Data are unitless.

Table 5 shows kinome-wide screening of a panel of inhibitors. Compounds were screened using the SelectScreen™ platform developed by Life Technologies. Z'lyte (a, measures kinase activity), Adapta (b, measures kinase activity) and Lantha assays (c, measures ATP binding) were performed. Inhibition data are represented in a heat map format.

Table 6 shows comparison of selectivity of 13, 1NA-PP1 and 1NM-PP1. All kinases for which >40% inhibition was observed in a kinome wide Z'lyte screen (Life Technologies) are shown. Legend for Tables 5-6: <40% inhibition (gray); 40%-80% inhibition (white);  $\geq$ 80% inhibition (diagonal stripes).

TABLE 5a

Conc Compound			1000 nM 3	1000 nM 4	1000 nM 9	1000 nM 13	1000 nM 20
ABL1	Activity	Km app	7	5	4	2	1
ABL1 E255K	Activity	Km app	14	8	5	3	8
ABL1 G250E	Activity	Km app	3	1	0	-3	1
ABL1 T315I	Activity	Km app	-1	3	4	-2	-4
ABL1 Y253F	Activity	Km app	17	16	12	10	12
ABL2 (Arg)	Activity	Km app	17	15	11	5	8
ACVR1B (ALK4)	Activity	Km app	13	8	5	2	-1
ADRBK1 (GRK2)	Activity	Km app	23	22	21	18	13
ADRBK2 (GRK3)	Activity	Km app	0	0	1	-1	0
AKT1 (PKB alpha)	Activity	Km app	-4	-1	0	0	-4
AKT2 (PKB beta)	Activity	Km app	5	3	4	5	3
AKT3 (PKB gamma)	Activity	Km app	-1	2	5	5	1
ALK	Activity	Km app	5	7	2	0	1
AMPK A1/B1/G1	Activity	Km app	14	20	29	28	14
AMPK A2/B1/G1	Activity	Km app	12	14	20	19	7
AURKA (Aurora A)	Activity	Km app	7	4	9	3	12

TABLE 5a-continued

AURKB (Aurora B)	Activity	Km app	9	9	9	5	27
AURKC (Aurora C)	Activity	Km app	8	8	7	6	25
AXL	Activity	Km app	9	6	1	1	9
BLK	Activity	Km app	48	34	20	10	42
BMX	Activity	Km app	8	8	68	9	20
BRAF	Activity	100	35	5	7	4	6
BRAF V599E	Activity	100	8	13	17	21	9
BRSK1 (SAD 1)	Activity	Km app	11	14	13	9	17
BTK	Activity	Km app	44	36	42	1	17
CAMK1D (CaMKI delta)	Activity	Km app	21	22	18	20	16
CAMK2A (CaMKII alpha)	Activity	Km app	-2	1	2	5	1
CAMK2B (CaMKII beta)	Activity	Km app	6	6	11	1	8
CAMK2D (CaMKII delta)	Activity	Km app	16	15	8	10	8
CAMK4 (CaMKIV)	Activity	Km app	9	8	10	11	7
CDC42 BPA (MRCKA)	Activity	Km app	12	16	22	24	20
CDC42 BPB (MRCKB)	Activity	Km app	1	-1	-3	-7	-1
CDK1/cyclin B	Activity	Km app	10	2	7	4	1
CDK2/cyclin A	Activity	Km app	9	5	16	11	0
CDK5/p25	Activity	Km app	13	9	21	12	9

CDK5/p35	Activity	Km app	18	8	24	7	5
CHEK1 (CHK1)	Activity	Km app	11	-3	-5	-7	-11
CHEK2 (CHK2)	Activity	Km app	0	-3	2	2	17
CLK1	Activity	Km app	8	7	12	7	7
CLK2	Activity	Km app	0	-1	0	-1	-3
CLK3	Activity	Km app	6	8	7	6	7
CSF1R (FMS)	Activity	Km app	7	10	7	4	5
CSK	Activity	Km app	22	9	9	8	12
CSNK1A1 (CK1 alpha 1)	Activity	Km app	20	12	6	14	0
CSNK1D (CK1 delta)	Activity	Km app	9	5	8	15	4
CSNK1E (CK1 epsilon)	Activity	Km app	18	6	10	40	7
CSNK1G1 (CK1 gamma 1)	Activity	Km app	3	4	4	1	15
CSNK1G2 (CK1 gamma 2)	Activity	Km app	7	7	8	3	36
CSNK1G3 (CK1 gamma 3)	Activity	Km app	6	11	10	9	31
CSNK2A1 (CK2 alpha 1)	Activity	Km app	17	18	5	14	12
CSNK2A2 (CK2 alpha 2)	Activity	Km app	6	-2	-1	1	-5
DAPK3 (ZIPK)	Activity	Km app	1	2	3	0	3
DCAMKL2 (DCK2)	Activity	Km app	7	5	6	2	8
DNA-PK	Activity	Km app	68	27	15	12	12

TABLE 5a-continued

DYRK1A	Activity	Km app	-1	-1	1	-5	-3
DYRK1B	Activity	Km app	-1	2	2	-1	2
DYRK3	Activity	Km app	3	2	23	0	4
DYRK4	Activity	Km app	1	3	3	3	2
EEF2K	Activity	Km app	5	7	8	7	5
EGFR (ErbB1)	Activity	Km app	50	26	8	-5	71
EGFR (ErbB1) L858R	Activity	Km app	61	36	23	1	71
EGFR (ErbB1) L861Q	Activity	Km app	46	46	18	1	71
EGFR (ErbB1) T790M	Activity	Km app	36	27	12	4	12
EGFR (ErbB1) T790M L858R	Activity	Km app	64	39	19	10	23
EPHA1	Activity	Km app	33	14	14	15	11
EPHA2	Activity	Km app	7	5	6	4	5
EPHA4	Activity	Km app	19	8	8	7	9
EPHA5	Activity	Km app	22	11	11	5	7
EPHA8	Activity	Km app	23	7	9	9	6
EPHB1	Activity	Km app	12	6	6	5	7
EPHB2	Activity	Km app	21	12	15	14	12
EPHB3	Activity	Km app	40	10	18	6	8
EPHB4	Activity	Km app	14	11	11	6	9

ERBB2 (HER2)	Activity	Km app	40	23	19	16	55
ERBB4 (HER4)	Activity	Km app	70	9	15	5	71
FER	Activity	Km app	13	9	7	7	10
FES (FPS)	Activity	Km app	8	10	2	12	11
FGFR1	Activity	Km app	24	53	22	15	1
FGFR2	Activity	Km app	18	3	4	3	8
FGFR3	Activity	Km app	20	10	4	6	9
FGFR3 K650E	Activity	Km app	28	2	6	-2	9
FGFR4	Activity	Km app	17	8	5	6	-1
FGR	Activity	Km app	79	16	20	6	30
FLT1 (VEGFR1)	Activity	Km app	3	1	2	-2	2
FLT3	Activity	Km app	29	0	6	-7	31
FLT3 D835Y	Activity	Km app	34	16	42	8	15
FLT4 (VEGFR3)	Activity	Km app	28	33	13	9	41
FRAP1 (mTOR)	Activity	Km app	24	-2	-8	-8	-10
FRK (PTK5)	Activity	Km app	25	9	8	5	9
FYN	Activity	Km app	17	9	10	6	-3
GRK4	Activity	Km app	10	10	5	-2	0
GRK5	Activity	Km app	1	-3	-3	-3	2
GRK6	Activity	Km app	15	16	10	7	5

TABLE 5a-continued

GRK7	Activity	Km app	-1	0	-1	-3	0
GSK3A (GSK3 alpha)	Activity	Km app	3	5	5	-3	-3
GSK3B (GSK3 beta)	Activity	Km app	3	0	3	-5	-3
HCK	Activity	Km app	17	4	6	5	11
HIPK1 (Myak)	Activity	Km app	8	5	4	4	4
HIPK2	Activity	Km app	11	6	4	4	4
HIPK3 (YAK1)	Activity	Km app	5	6	4	3	5
HIPK4	Activity	Km app	6	6	9	4	7
IGF1R	Activity	Km app	5	4	0	-7	2
IKBKB (IKK beta)	Activity	Km app	16	17	18	16	12
IKBKE (IKK epsilon)	Activity	Km app	16	20	15	14	10
INSR	Activity	Km app	-2	2	3	2	0
INSRR (IRR)	Activity	Km app	9	10	9	9	11
IRAK4	Activity	Km app	4	1	2	-6	-2
ITK	Activity	Km app	3	-6	-4	-4	-7
JAK1	Activity	Km app	4	8	14	16	11

JAK2	Activity	Km app	7	5	5	4	2
JAK2 JH1 JH2	Activity	Km app	1	-1	-6	-4	-1
JAK2 JH1 JH2 V617F	Activity	Km app	2	1	-3	-4	-2
JAK3	Activity	Km app	23	72	14	10	12
KDR (VEGFR2)	Activity	Km app	6	-14	-17	-18	16
KIT	Activity	Km app	17	19	12	7	6
KIT T670I	Activity	Km app	10	7	8	7	3
LCK	Activity	Km app	48	-1	-13	-18	11
LTK (TYK1)	Activity	Km app	1	0	-4	-5	1
LYN A	Activity	Km app	34	9	12	8	28
LYN B	Activity	Km app	39	16	15	14	30
MAP2K1 (MEK1)	Activity	100	31	7	3	5	0
MAP2K2 (MEK2)	Activity	100	49	12	11	10	7
MAP2K6 (MKK6)	Activity	100	5	8	11	13	16
MAP3K8 (COT)	Activity	100	33	2	0	0	0
MAP3K9 (MLK1)	Activity	Km app	8	5	4	1	21
MAP4K2 (GCK)	Activity	Km app	-16	2	7	11	3
MAP4K4 (HGK)	Activity	Km app	15	14	16	18	21
MAP4K5 (KHS1)	Activity	Km app	22	17	48	20	36

TABLE 5a-continued

MAPK1 (ERK2)	Activity	Km app	3	6	3	2	2
MAPK10 (JNK3)	Activity	100	6	13	2	7	10
MAPK11 (p38 beta)	Activity	Km app	9	11	9	10	9
MAPK12 (p38 gamma)	Activity	Km app	9	13	10	9	14
MAPK13 (p38 delta)	Activity	Km app	2	6	6	5	6
MAPK14 (p38 alpha)	Activity	100	21	20	22	24	22
MAPK14 (p38 alpha) Direct	Activity	Km app	-1	4	9	12	10
MAPK3 (ERK1)	Activity	Km app	15	31	13	10	14
MAPK8 (JNK1)	Activity	100	17	21	24	21	19
MAPK9 (JNK2)	Activity	100	5	7	8	8	7
MAPKAPK2	Activity	Km app	1	2	4	6	5
MAPKAPK3	Activity	Km app	5	6	5	3	5
MAPKAPK5 (PRAK)	Activity	Km app	4	1	4	7	7
MARK1 (MARK)	Activity	Km app	-1	0	2	2	-4
MARK2	Activity	Km app	1	2	4	5	1
MARK3	Activity	Km app	6	8	6	2	5
MARK4	Activity	Km app	2	4	3	-1	-1
MATK (HYL)	Activity	Km app	3	5	6	5	4
MELK	Activity	Km app	16	28	29	15	32

MERTK (cMER)	Activity	Km app	9	4	8	17	48
MET (cMet)	Activity	Km app	-4	27	10	8	0
MET M1250T	Activity	Km app	7	7	4	2	6

MINK1	Activity	Km app	31	17	23	19	22
MKNK1 (MNK1)	Activity	Km app	5	0	-4	-5	16
MST1R (RON)	Activity	Km app	13	11	9	5	9
MST4	Activity	Km app	8	13	21	7	30
MUSK	Activity	Km app	15	12	11	18	1
MYLK2 (skMLCK)	Activity	Km app	2	2	5	0	2
NEK1	Activity	Km app	14	-5	7	4	10
NEK2	Activity	Km app	-1	-4	-10	0	-8
NEK4	Activity	Km app	8	10	17	19	15
NEK6	Activity	Km app	4	5	10	12	4
NEK7	Activity	Km app	7	8	8	8	6
NEK9	Activity	Km app	15	12	13	12	12
NTRK1 (TRKA)	Activity	Km app	37	-3	21	17	48
NTRK2 (TRKB)	Activity	Km app	16	4	23	12	-1
NTRK3 (TRKC)	Activity	Km app	16	-5	18	-6	-8
PAK1	Activity	Km app	12	12	10	13	16

TABLE 5a-continued

PAK2 (PAK65)	Activity	Km app	18	12	12	13	10
PAK3	Activity	Km app	4	3	6	6	2
PAK4	Activity	Km app	-5	-8	-7	-6	-5
PAK6	Activity	Km app	7	10	10	8	5
PAK7 (KIAA1264)	Activity	Km app	9	13	13	0	11
PASK	Activity	Km app	11	9	8	7	7
PDGFRA (PDGFR alpha)	Activity	Km app	13	6	5	4	16
PDGFRA D842V	Activity	Km app	5	3	0	3	9
PDGFRA T674I	Activity	Km app	11	11	-2	4	11
PDGFRA V561D	Activity	Km app	19	12	5	1	27
PDGFRB (PDGFR beta)	Activity	Km app	16	10	5	5	11
PDK1	Activity	100	5	11	11	11	11
PDK1 Direct	Activity	Km app	1	-1	3	0	-5
PHKG1	Activity	Km app	8	8	8	10	5
PHKG2	Activity	Km app	1	6	4	-1	7

PIM1	Activity	Km app	16	12	11	12	10
PIM2	Activity	Km app	3	1	2	-1	-3
PKN1 (PRK1)	Activity	Km app	15	17	18	12	9
PLK1	Activity	Km app	-7	-3	-6	-3	4
PLK2	Activity	Km app	13	8	7	1	7
PLK3	Activity	Km app	2	4	0	-2	3
PRKACA (PKA)	Activity	Km app	-1	0	-1	-4	-2
PRKCA (PKC alpha)	Activity	Km app	8	15	19	17	18
PRKCB1 (PKC beta I)	Activity	Km app	-1	11	10	8	5
PRKCB2 (PKC beta II)	Activity	Km app	9	13	7	4	4
PRKCD (PKC delta)	Activity	Km app	14	18	20	16	17
PRKCE (PKC epsilon)	Activity	Km app	12	16	21	19	10
PRKCG (PKC gamma)	Activity	Km app	18	13	22	19	14
PRKCH (PKC eta)	Activity	Km app	28	6	25	24	10
PRKCI (PKC iota)	Activity	Km app	10	13	13	13	10
PRKCN (PKD3)	Activity	Km app	8	7	12	16	11
PRKCQ (PKC theta)	Activity	Km app	9	11	13	13	12
PRKCZ (PKC zeta)	Activity	Km app	0	5	5	8	7
PRKD1 (PKC mu)	Activity	Km app	8	10	17	18	10

PRKD2 (PKD2)	Activity	Km app	11	11	12	19	16
PRKG1	Activity	Km app	1	2	3	-1	4
PRKG2 (PKG2)	Activity	Km app	0	0	-1	0	0
PRKX	Activity	Km app	3	5	3	1	5
PTK2 (FAK)	Activity	Km app	7	7	8	7	6
PTK2B (FAK2)	Activity	Km app	5	3	2	1	1
PTK6 (Brk)	Activity	Km app	68	-3	30	27	74

TABLE 5a-continued

RAF1 (cRAF) Y340D Y341D	Activity	100	19	7	7	7	18
RET	Activity	Km app	41	11	12	8	40
RET V804L	Activity	Km app	4	3	6	2	13
RET Y791F	Activity	Km app	44	9	10	8	41
ROCK1	Activity	Km app	2	0	0	-3	-1
ROCK2	Activity	Km app	18	21	19	14	24
ROS1	Activity	Km app	7	23	5	2	5
RPS6KA1 (RSK1)	Activity	Km app	1	1	1	-2	5
RPS6KA2 (RSK3)	Activity	Km app	2	8	3	0	12
RPS6KA3 (RSK2)	Activity	Km app	4	4	4	4	4
RPS6KA4 (MSK2)	Activity	Km app	11	7	8	6	6
RPS6KA5 (MSK1)	Activity	Km app	7	8	6	3	7
RPS6KA6 (RSK4)	Activity	Km app	24	21	19	5	53
RPS6KB1 (p70S6K)	Activity	Km app	8	8	14	13	10
SGK (SGK1)	Activity	Km app	10	4	6	-1	2
SGK2	Activity	Km app	9	10	7	5	11
SGKL (SGK3)	Activity	Km app	5	5	5	3	6
SNF1LK2	Activity	Km app	3	5	2	1	3
SRC	Activity	Km app	40	3	1	-6	20

SRC N1	Activity	Km app	53	8	13	4	19
SRMS (Srm)	Activity	Km app	///	19	11	10	13
SRPK1	Activity	Km app	2	3	4	4	2
SRPK2	Activity	Km app	13	13	11	12	9
STK22B (TSSK2)	Activity	Km app	5	3	0	0	6
STK22D (TSSK1)	Activity	Km app	3	6	10	12	11
STK23 (MSSK1)	Activity	Km app	7	7	9	6	8
STK24 (MST3)	Activity	Km app	13	13	9	10	13
STK25 (YSK1)	Activity	Km app	10	8	10	0	9
STK3 (MST2)	Activity	Km app	-7	-9	-8	-9	-13
STK4 (MST1)	Activity	Km app	12	5	3	2	3
SYK	Activity	Km app	-7	-6	-6	-8	-2
TAOK2 (TAO1)	Activity	Km app	6	4	6	2	2
TBK1	Activity	Km app	5	-2	0	11	-5
TEK (Tie2)	Activity	Km app	-3	-9	-8	-7	-10
TXK	Activity	Km app	80	66	///	7	62
TYK2	Activity	Km app	-2	-2	-2	-5	-5
TYRO3 (RSE)	Activity	Km app	20	13	10	8	14
YES1	Activity	Km app	64	15	17	8	43
ZAP70	Activity	Km app	12	13	10	11	10

TABLE 5b

Conc Compound			1000 nM 3	1000 nM 4	1000 nM 9	1000 nM 13	1000 nM 20
CAMK1 (CaMK1)	Activity	100	-6	-11	-4	-8	-20
CDK7/cyclin H/MNAT1	Activity	Km app	-7	-1	20	-10	10
CDK9/cyclin T1	Activity	Km app	23	14	-2	9	13
CHUK (IKK alpha)	Activity	Km app	1	5	12	6	2
DAPK1	Activity	Km app	-1	5	9	-5	-5
GSG2 (Haspin)	Activity	Km app	21	6	13	12	9
IRAK1	Activity	Km app	0	8	15	14	13
LRRK2	Activity	Km app	7	7	0	-2	21

LRRK2 G2019S	Activity	Km app	-4	-13	-17	-7	-5
NUAK1 (ARK5)	Activity	Km app	9	15	22	14	12
PI4KA (PI4K alpha)	Activity	10	12	-6	0	2	9
PI4KB (PI4K beta)	Activity	Km app	37	13	29	13	9
PIK3C2A (PI3K-C2 alpha)	Activity	Km app	-3	9	3	-4	8
PIK3C2B (PI3K-C2 beta)	Activity	100	26	2	3	-1	13
PIK3C3 (hVPS34)	Activity	Km app	-6	3	-1	-5	0
PIK3CA/PIK3R1 (p110 alpha/p85 alpha)	Activity	Km app	51	-1	7	2	9
PIK3CD/PIK3R1 (p110 delta/p85 alpha)	Activity	Km app	66	7	18	-1	4
PIK3CG (p110 gamma)	Activity	Km app	48	11	-3	-2	5
SPHK1	Activity	Km app	-16	-2	3	8	7
SPHK2	Activity	100	9	-4	-7	-14	4

TABLE 5c

Conc Compound		1000 nM 3	1000 nM 4	1000 nM 9	1000 nM 13	1000 nM 20
ACVR1 (ALK2)	Binding	///	10	12	3	-5
ACVR2B	Binding	20	19	4	8	-11
BMPRI1A (ALK3)	Binding	37	5	4	4	12
CAMKK1 (CAMKKA)	Binding	1	2	1	-10	0
CAMKK2 (CaMKK beta)	Binding	8	8	5	-1	5
CDK8/cyclin C	Binding	23	27	20	9	4
CDK9/cyclin K	Binding	11	3	10	6	6
CLK4	Binding	35	7	19	7	5
DDR1	Binding	1	1	-3	0	2
DDR2	Binding	7	2	-1	-4	5
DMPK	Binding	5	3	3	1	10
EPHA3	Binding	2	8	-3	5	-3

TABLE 5c-continued

EPHA7	Binding	-3	-5	-1	1	10
KIT V654A	Binding	12	5	1	1	8
LIMK1	Binding	21	4	2	-6	-4
LIMK2	Binding	14	12	8	9	10
MAP2K1 (MEK1) S218D S222D	Binding	98	4	5	1	1
MAP2K3 (MEK3)	Binding	12	18	9	0	24
MAP2K6 (MKK6) S207E T211E	Binding	64	3	4	6	1
MAP3K10 (MLK2)	Binding	20	3	1	2	9
MAP3K11 (MLK3)	Binding	5	2	4	-2	9
MAP3K14 (NIK)	Binding	-6	-13	-9	-12	-9
MAP3K2 (MEKK2)	Binding	13	0	8	1	6
MAP3K3 (MEKK3)	Binding	13	7	10	8	10
MAP3K5 (ASK1)	Binding	15	-11	-4	-8	-4
MAP3K7/MAP3K7IP1 (TAK1-TAB1)	Binding	2	2	2	-2	6
MKNK2 (MNK2)	Binding	19	2	2	2	13

MLCK (MLCK2)	Binding	1	9	10	6	14
MYLK (MLCK)	Binding	3	1	0	0	0
NLK	Binding	56	3	-1	-4	-10
RIPK2	Binding	77	6	4	2	8
SLK	Binding	4	4	4	5	5
STK16 (PKL12)	Binding	2	3	6	1	3
STK17A (DRAK1)	Binding	1	0	-2	7	2
STK33	Binding	3	7	-5	-6	5
TAOK3 (JIK)	Binding	1	-5	-2	-3	4
TEC	Binding	15	3	14	14	5
TGFBR1 (ALK5)	Binding	65	7	10	7	3
TNK2 (ACK)	Binding	11	3	5	4	10
TTK	Binding	30	12	22	16	25
WEE1	Binding	20	1	1	-2	-14
WNK2	Binding	55	9	7	9	15
ZAK	Binding	75	27	33	4	8

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Table 6 Comparison of selectivity of 13, 1NA-PP1 and 1NM-PP1. All kinases for which >40% inhibition was observed in a kinome wide Z'lyte screen (Life Technologies) are shown.

TABLE 6

Kinase tested	13	1NA-PP1	1NM-PP1
BMX	9	26	52
BTK	1	49	61
CSF1R (FMS)	4	40	28
CSNK1E (CK1 epsilon)	40		
EGFR (ErbB1) L858R	1	40	29
EGFR (ErbB1) T790M	4	48	38
EGFR (ErbB1) T790M L858R	10	65	53
EPHA1	15		
EPHA2	4	75	43
EPHA4	7		43
EPHA5	5		56
EPHA8	9	67	51
EPHB1	5	63	44
EPHB2	14	78	58
EPHB3	6	48	35
EPHB4	6	73	62
FGR	6	71	42

FRK (PTK5)	5		47
FYN	6	45	37
HCK	5	59	31
LCK	18	52	35
LYN A	8		55
LYN B	14		55
MAP4K4 (HGK)	18		56
MAP4K5 (KHS1)	20	69	31
MINK1	19		52
PRKACA (PKA)	4	12	41
PRKCN (PKD3)	16	69	65
PRKD1 (PKC mu)	18	1	56
PRKD2 (PKD2)	19	59	
PRKGI	1	75	0
PTK6 (Brk)	27		55
RET	8	79	65
RET Y791F	8		70
SRC	6	65	27
SRMS (Srm)	10	61	7
YES1	8	54	42

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TABLE 7

Compound	c-Src variant IC <sub>50</sub> (nM)		
	ES1	ES2	ES3
3	111	63	131
9	150	207	424
13	338	67	29

IC<sub>50</sub> values of a panel of electrophilic inhibitors against c-Src-ES variants with second-site mutations (ES1 = T338C; ES2 = T338C/V323A; ES3 = T338C/V323S).

#### XIV. Kit

In some other embodiments, the present invention provides a kit comprising, a recombinant kinase described herein (see section (V) above) or a nucleic acid described herein (see section IX) and instructions for using the kit. The instructions for using the kit describe the steps set forth in a method provided herein (see section X, XI and XII).

In some embodiments, the present invention provides a kit for testing for inhibition of kinase activity comprising a heterocyclic compound, wherein the heterocyclic compound comprises two or more fused rings and an electrophilic substituent, wherein at least one of the two or more fused rings comprises a nitrogen atom, and a cysteine substituted kinase wherein a gatekeeper amino acid residue within an ATP binding site of the kinase is replaced with a cysteine residue.

#### XV. Examples

Chemical Synthesis. Reactions were performed in flame dried flasks under argon with magnetic stirring. All <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded on a Varian Innova 400 spectrometer and referenced to solvent peaks. <sup>1</sup>H chemical shifts are reported in δ (ppm) as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) or br (broad). Low resolu-

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tion mass spectra (LC/ESI-MS) were recorded on a Waters Micromass ZQ equipped with a Waters 2695 Separations Module and a XTerra MS C18 3.5 mm column (Waters). RP-HPLC was performed on a Varian ProStar solvent delivery system equipped with a Zorbax 300-SS C18 column using  $\text{CH}_3\text{CN}/\text{H}_2\text{O}/0.1\%$  TFA (1-100% gradient) and monitoring at 260 nm.

## EXAMPLE 1

## Preparation of 3-(3-aminophenyl)-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine (22)

This compound was prepared in a similar procedure to that used for (18).

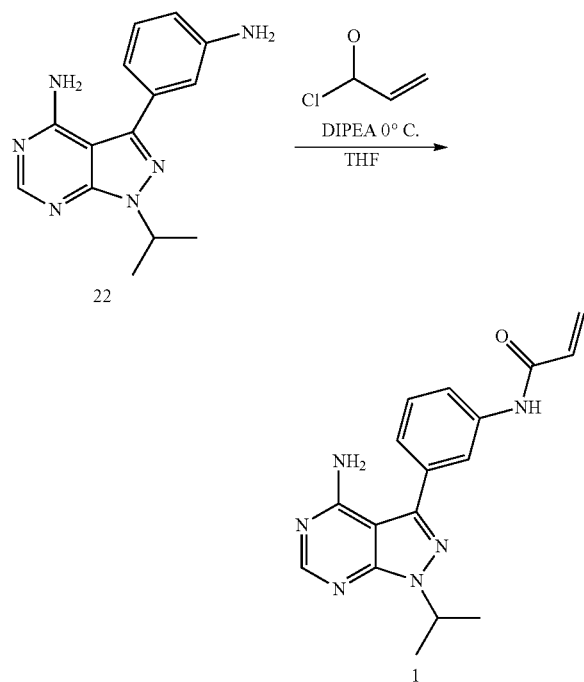
## EXAMPLE 2

## Preparation of 3-(4-aminophenyl)-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine (23)

This compound was prepared in a similar procedure to that used for (18).

## EXAMPLE 3

## Preparation of N-(3-(4-amino-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-3-yl)phenyl)acrylamide (1)

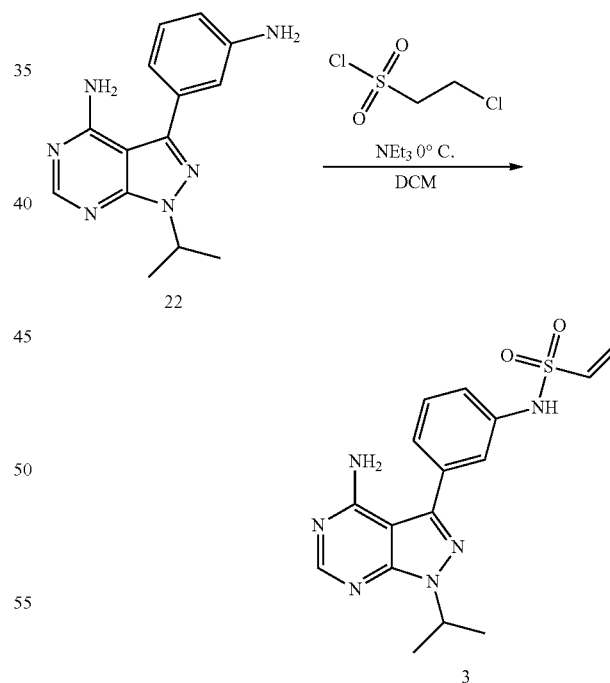


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A solution of tetrahydrofuran (20 mL), compound 22 (219 mg, 0.817 mmol) and diisopropylethylamine (156  $\mu\text{L}$ , 0.895 mmol) was cooled to 0° C. Acryloyl chloride (67  $\mu\text{L}$ , 0.828 mmol) was added and the reaction was allowed to proceed for 1 hour and afterwards concentrated in vacuo. The residue was dissolved in dichloromethane (20 mL) and washed with saturated sodium bicarbonate (20 mL). The aqueous layer was extracted with dichloromethane (2×20 mL). The combined organic layers were dried with  $\text{MgSO}_4$ , filtered and concentrated in vacuo. The product was purified by preparative RP-HPLC and lyophilized (70 mg, 26% yield):  $^1\text{H}$  NMR (400 MHz, DMSO)  $\delta$  10.40 (s, 1H), 8.38 (s, 1H), 8.04 (s, 1H), 7.73 (d, J=8.0, 1H), 7.52 (t, J=7.9, 1H), 7.39 (d, J=7.7, 1H), 6.47 (dd, J=17.0, 10.1, 1H), 6.29 (dd, J=17.0, 2.0, 1H), 5.80 (dd, J=10.1, 2.0, 1H), 5.11 (hept, J=6.6, 1H), 1.51 (d, J=6.7, 6H);  $^{13}\text{C}$  NMR (100 MHz, DMSO)  $\delta$  163.47, 154.87, 151.90, 150.94, 144.73, 139.53, 132.54, 131.68, 129.88, 127.31, 123.42, 119.96, 119.22, 96.91, 48.80, 21.74;  $[\text{M}+\text{H}]^+$  calculated for  $\text{C}_{17}\text{H}_{18}\text{N}_6\text{O}$  323.1, found 323.5.

## EXAMPLE 4

## Preparation of N-(3-(4-amino-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-3-yl)phenyl)ethanesulfonamide (3)



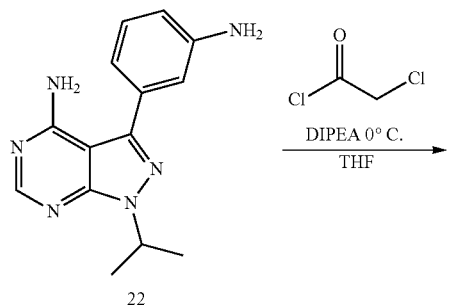
A solution of dichloromethane (2 mL), compound 22 (20 mg, 0.075 mmol) and triethylamine (11  $\mu\text{L}$ , 0.079 mmol) was cooled to 0° C. 2-chloro-1-ethane sulfonyl chloride (7  $\mu\text{L}$ , 0.067 mmol) was added and the reaction was allowed to proceed for 1 hour prior to addition of saturated sodium bicarbonate (10 mL) and extraction with dichloromethane (3×10 mL). The combined organic layers were dried with

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MgSO<sub>4</sub>, filtered and concentrated in vacuo. The product was purified by preparative RP-HPLC and lyophilized (5.6 mg, 23% yield): <sup>1</sup>H NMR (400 MHz, DMSO) δ 10.22 (s, 1H), 8.31 (s, 1H), 7.49 (t, J=7.9, 1H), 7.45 (s, 1H), 7.38 (d, J=7.8, 1H), 7.27 (d, J=7.3, 1H), 6.86 (dd, J=16.4, 10.0, 1H), 6.17 (d, J=16.4, 1H), 6.08 (d, J=9.9, 1H), 5.08 (hept, J=6.7, 1H), 1.50 (d, J=6.7, 6H). <sup>13</sup>C NMR (100 MHz, DMSO) δ 155.01, 151.96, 151.19, 144.29, 138.50, 136.32, 133.09, 130.22, 127.79, 123.73, 120.09, 119.20, 96.94, 48.85, 21.73; [M+H]<sup>+</sup> calculated for C<sub>16</sub>H<sub>18</sub>N<sub>6</sub>O<sub>2</sub>S 359.1, found 359.4.

## EXAMPLE 5

Preparation of N-(3-(4-amino-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-3-yl)phenyl)-2-chloroacetamide (6)



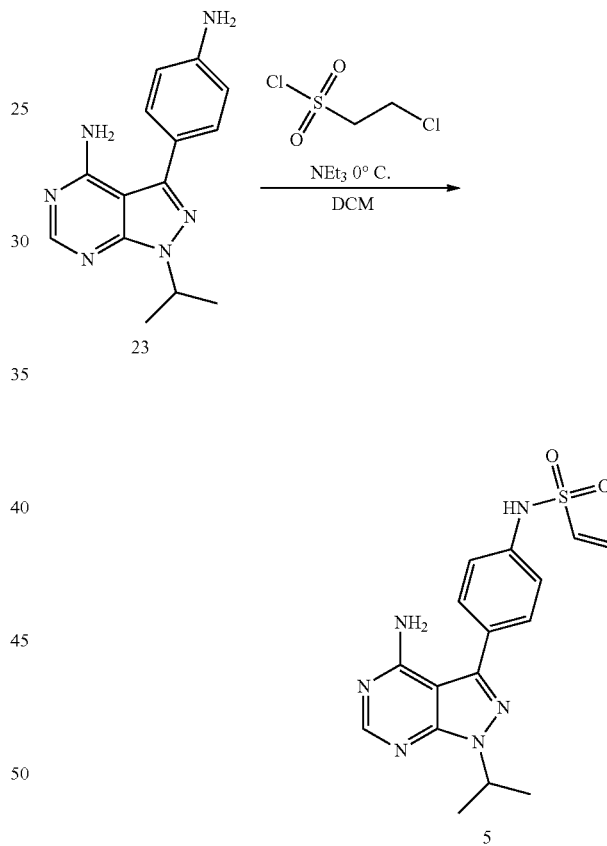
A solution of THF (20 mL), compound 22 (200 mg, 0.75 mmol) and DIPEA (143 μL, 0.821 mmol) was cooled to 0° C. Chloroacetylchloride (54 μL, 0.67 mmol) was added and the reaction was allowed to proceed for 1 hour and afterwards concentrated in vacuo. The residue was dissolved in dichloromethane (20 mL) and washed with saturated sodium bicarbonate (20 mL). The aqueous layer was extracted with dichloromethane (2×20 mL). The combined organic layers were dried with MgSO<sub>4</sub>, filtered and concentrated in vacuo. The product was purified by preparative RP-HPLC and lyophilized (30.4 mg, 13% yield): <sup>1</sup>H NMR (400 MHz, DMSO)

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δ 10.57 (s, 1H), 8.41 (s, 1H), 7.95 (s, 1H), 7.67 (d, J=7.6, 1H), 7.53 (t, J=7.9, 1H), 7.41 (d, J=7.7, 1H), 5.11 (hept, J=6.7, 1H), 4.30 (s, 2H), 1.51 (d, J=6.7, 6H); <sup>13</sup>C NMR (100 MHz, DMSO) δ 165.06, 154.82, 151.89, 150.90, 144.68, 139.03, 132.62, 128.28, 123.81, 120.02, 119.24, 96.92, 48.84, 43.55, 21.77; [M+H]<sup>+</sup> calculated for C<sub>16</sub>H<sub>17</sub>ClN<sub>6</sub>O 345.1, found 345.4.

## EXAMPLE 6

Preparation of N-(4-(4-amino-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-3-yl)phenyl)ethanesulfonamide (5)

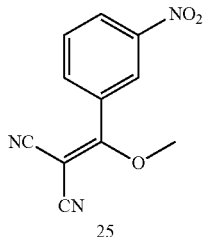
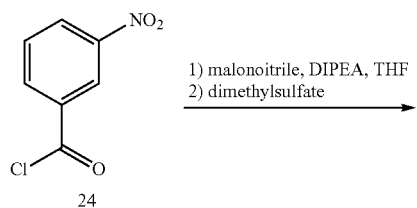


A solution of dichloromethane (5 mL), compound 23 (45 mg, 0.168 mmol) and triethylamine (71 μL, 0.509 mmol) was cooled to 0° C. 2-chloro-1-ethane sulfonyl chloride (16 μL, 0.148 mmol) was added and the reaction was allowed to proceed for 1 hour prior to addition of saturated sodium bicarbonate (10 mL) and extraction with dichloromethane (2×10 mL). The combined organic layers were dried with MgSO<sub>4</sub>, filtered and concentrated in vacuo. The product was purified by preparative RP-HPLC and lyophilized (8.7 mg,

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16% yield):  $^1\text{H}$  NMR (400 MHz, DMSO)  $\delta$  10.30 (s, 1H), 8.36 (s, 1H), 7.60 (d,  $J=8.5$ , 2H), 7.31 (d,  $J=8.6$ , 2H), 6.86 (dd,  $J=16.4$ , 9.9, 1H), 6.21 (d,  $J=16.4$ , 1H), 6.09 (d,  $J=9.9$ , 1H), 5.08 (hept,  $J=6.7$ , 2H), 1.49 (d,  $J=6.7$ , 6H);  $^{13}\text{C}$  NMR (100 MHz, DMSO)  $\delta$  155.44, 152.03, 151.64, 144.22, 138.54, 136.17, 129.25, 127.98, 127.41, 119.52, 96.95, 48.66, 21.75;  $[\text{M}+\text{H}]^+$  calculated for  $\text{C}_{16}\text{H}_{18}\text{N}_6\text{O}_2\text{S}$  359.1, found 359.5.

## EXAMPLE 7

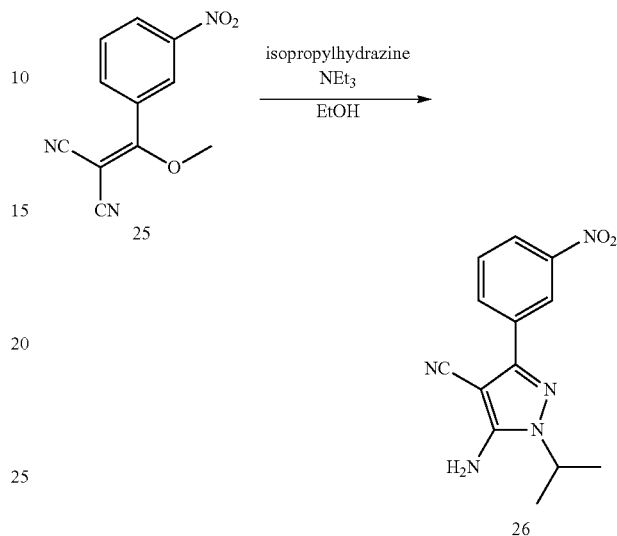
Preparation of  
2-(methoxy(3-nitrophenyl)methylene)malononitrile  
(25)

A solution of 3-nitrobenzoylchloride (25 g, 134 mmol), malononitrile (9.74 g, 147 mmol) and THF (140 mL) was cooled to  $0^\circ\text{C}$ . DIPEA (59 mL, 335 mmol) was added dropwise and the reaction was allowed to warm to room temperature and proceed for 2 hours. Afterwards, dimethylsulfate (38 mL, 399 mmol) was added and the temperature was raised to  $70^\circ\text{C}$  for 4 hours. Next, the reaction mixture was brought to room temperature and allowed to proceed for an additional 12 hours. EtOAc (200 mL) was added to the reaction mixture in addition to brine (200 mL). The organic and aqueous layers were separated and the aqueous layer was extracted with EtOAc (4x25 mL). The combined organic layers were dried with  $\text{MgSO}_4$  and concentrated in vacuo. The material was purified over a silica column using chloroform:hexane (90:10) initially and eluted with pure chloroform. After concentrating the fractions containing the product, a yellow oil was triturated with diethyl ether to yield a solid (11.3 g, 37% yield):  $^1\text{H}$  NMR (400 MHz, DMSO)  $\delta$  8.63 (s, 1H), 8.51 (d,  $J=7.7$ , 1H), 8.16 (d,  $J=7.7$ , 1H), 7.94 (t,  $J=7.8$ , 1H), 3.93 (s, 3H);  $^{13}\text{C}$  NMR (100 MHz, DMSO)  $\delta$  183.66, 147.82, 135.23, 131.00, 129.55, 127.15, 124.08, 113.31, 111.87, 66.71, 61.87;  $[\text{M}+\text{H}]^+$  calculated for  $\text{C}_{11}\text{H}_7\text{N}_3\text{O}_3$  228.0, found 214.10 (product appears to hydrolyze during LC/MS analysis).

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## EXAMPLE 8

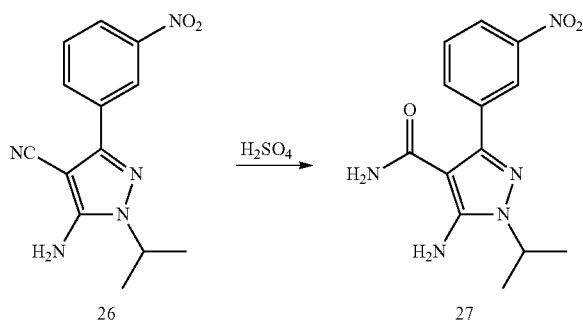
## 5-amino-1-isopropyl-3-(3-nitrophenyl)-1H-pyrazole-4-carbonitrile (26)



Compound 25 (5 g, 21.8 mmol), isopropylhydrazine hydrochloride (2.41 g, 21.8 mmol) (purchased from Ryan Scientific) and triethylamine (6.40 mL, 46.0 mmol) were allowed to react in ethanol (145 mL) at room temperature for 1 hr. After concentrating the reaction mixture, it was purified by silica chromatography using a chloroform/methanol solvent system (methanol gradient increased with time from 0-10%). The relevant fractions were concentrated in vacuo to yield a yellow powder (4.65 g, 79% yield):  $^1\text{H}$  NMR (400 MHz, DMSO)  $\delta$  8.59 (s, 1H), 8.24 (m, 2H), 7.78 (t,  $J=8.2$ , 1H), 6.82 (br, 2H), 4.53 (hept,  $J=6.5$ , 1H), 1.36 (d,  $J=6.5$ , 6H).  $^{13}\text{C}$  NMR (100 MHz, DMSO)  $\delta$  153.04, 148.76, 146.98, 133.97, 132.22, 131.19, 123.80, 120.37, 116.28, 70.74, 48.64, 22.02;  $[\text{M}+\text{H}]^+$  calculated for  $\text{C}_{13}\text{H}_{11}\text{N}_5\text{O}_2$  272.1, found 272.3.

## EXAMPLE 9

## Preparation of 5-amino-1-isopropyl-3-(3-nitrophenyl)-1H-pyrazole-4-carboxamide (27)

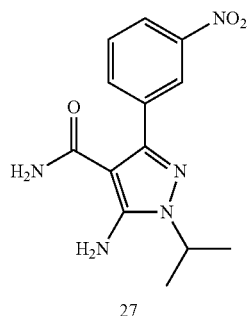


## 93

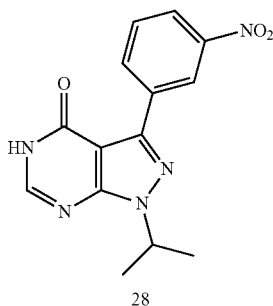
Compound 26 (100 mg, 0.369 mmol) was added to concentrated sulfuric acid (1 mL) and heated to 65° C. for 3 hours. Afterwards, the reaction mixture was poured into ice water and the pH was brought to 14 with 10 M NaOH. The aqueous material was extracted several times with dichloromethane. The combined organic layers were dried over MgSO<sub>4</sub>, filtered and concentrated to a solid (91 mg, 85% yield): <sup>1</sup>H NMR (400 MHz, DMSO) δ 8.32 (s, 1H), 8.21 (d, J=8.2, 1H), 7.98 (d, J=7.7, 1H), 7.69 (t, J=8.0, 1H), 6.20 (br, 2H), 4.51 (hept, 1H), 1.35 (d, J=6.5, 6H); <sup>13</sup>C NMR (100 MHz, DMSO) δ 166.17, 149.37, 147.65, 145.50, 135.63, 134.92, 129.69, 122.79, 122.43, 95.43, 47.11, 21.50; [M+H]<sup>+</sup> calculated for C<sub>13</sub>H<sub>15</sub>N<sub>5</sub>O<sub>3</sub> 290.1, found 290.0.

## EXAMPLE 10

Preparation of 1-isopropyl-3-(3-nitrophenyl)-1H-pyrazolo[3,4-d]pyrimidin-4(5H)-one (28)



formamide  
160° C.



Compound 27 (1 g, 3.46 mmol) was added to formamide (1.167 mL, 29.3 mmol) and heated to 160° C. for 40 hours. Afterwards, the reaction mixture was allowed to cool to room temperature and diluted into ice cold water. The mixture was filtered and a solid was collected (943 mg, 91% yield): <sup>1</sup>H NMR (400 MHz, DMSO) δ 12.37 (s, 1H), 9.32 (s, 1H), 8.81

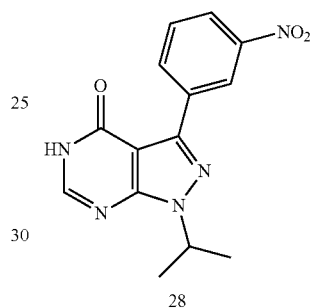
## 94

(d, J=7.8, 1H), 8.23 (d, J=8.1, 1H), 8.13 (s, 1H), 7.75 (t, J=8.0, 1H), 5.06 (hept, J=6.6, 1H), 1.52 (d, J=6.7, 6H); <sup>13</sup>C NMR (100 MHz, DMSO) δ 157.77, 152.48, 148.16, 148.06, 143.85, 133.58, 129.95, 123.05, 122.07, 103.02, 49.07, 21.74; [M+H]<sup>+</sup> calculated for C<sub>14</sub>H<sub>13</sub>N<sub>5</sub>O<sub>3</sub> 300.1, found 300.0.

## EXAMPLE 11

Preparation of 3-(1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-3-yl)aniline (28)

20



1) SOCl<sub>2</sub>, cat. DMF  
2) H<sub>2</sub>, Pd/C, NEt<sub>3</sub>,  
MeOH/EtOAc

25

30

35

40

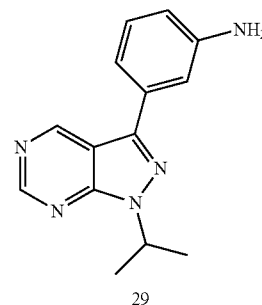
45

50

55

60

65



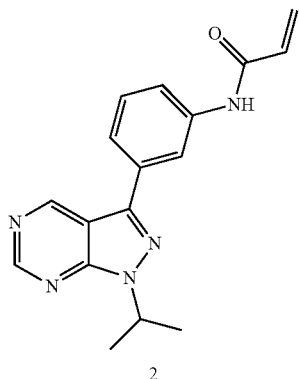
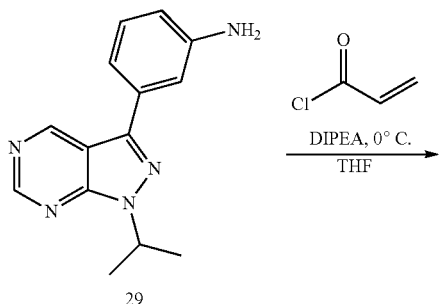
Compound 28 (2 g, 6.68 mmol) was mixed with thionyl chloride (12.5 mL, 171 mmol) and ten drops of DMF and heated to 80° C. for forty minutes. Afterwards, the reaction mixture was poured onto 300 mL of ice and the pH was adjusted to 8 with saturated sodium carbonate. The solution was extracted with dichloromethane (3×150 mL). The combined organic layers were dried over MgSO<sub>4</sub> and concentrated in vacuo (2.04 g, yield 96%). The resulting solid (1 g, 3.34 mmol) was dissolved in MeOH/EtOAc (30 mL/20 mL) and reacted with 10% Pd/C (540 mg) and triethylamine (466 μL, 3.34 mmol) under a H<sub>2</sub> atmosphere for 24 hours. The reaction mixture was filtered over celite and concentrated. The material was resuspended in dichloromethane (100 mL),

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which was washed with saturated sodium bicarbonate (2×100 mL) prior to drying with  $\text{MgSO}_4$  and concentrating in vacuo. The resulting solid was purified over silica using a dichloromethane/methanol (0-5%) solvent system (487 mg, 58% yield):  $^1\text{H}$  NMR (400 MHz, DMSO)  $\delta$  9.59 (s, 1H), 9.02 (s, 1H), 7.34 (s, 1H), 7.23 (d,  $J=7.6$ , 1H), 7.18 (t,  $J=7.7$ , 1H), 6.66 (d,  $J=7.7$ , 1H), 5.30 (br, 2H), 5.21 (kept,  $J=6.7$ , 1H), 1.56 (d,  $J=6.7$ , 6H);  $^{13}\text{C}$  NMR (100 MHz, DMSO)  $\delta$  154.60, 153.03, 151.87, 149.28, 143.17, 132.14, 129.59, 114.65, 114.32, 112.06, 111.85, 48.53, 21.72;  $[\text{M}+\text{H}]^+$  calculated for  $\text{C}_{14}\text{H}_{15}\text{N}_5$  254.1, found 254.0.

## EXAMPLE 12

Preparation of N-(3-(1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-3-yl)phenyl)acrylamide (2)

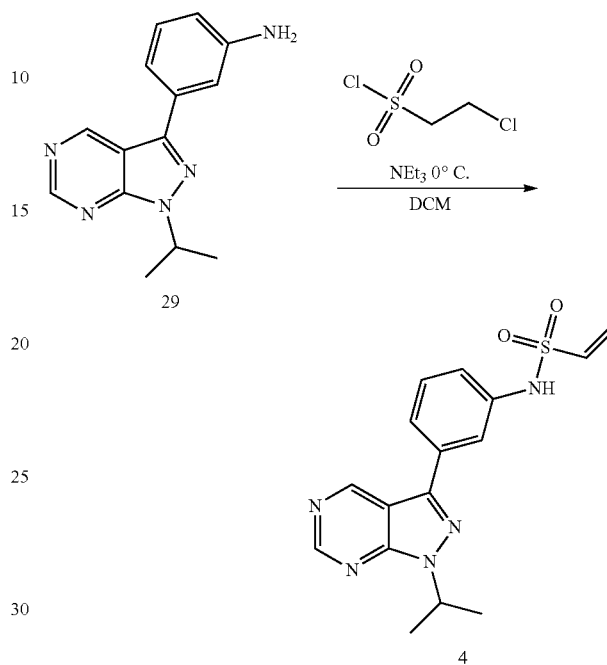


Compound 2 was prepared by the same procedure that was used for compound 1 (49% yield):  $^1\text{H}$  NMR (400 MHz, DMSO)  $\delta$  10.36 (s, 1H), 9.66 (s, 1H), 9.08 (s, 1H), 8.41 (t,  $J=1.8$ , 1H), 7.84 (m, 2H), 7.52 (t,  $J=7.9$ , 1H), 6.48 (dd,  $J=17.0$ , 10.0, 1H), 6.32 (dd,  $J=17.0$ , 2.0, 1H), 5.81 (dd,  $J=10.0$ , 2.0, 1H), 5.24 (kept,  $J=6.6$ , 1H), 1.58 (d,  $J=6.7$ , 6H);  $^{13}\text{C}$  NMR (100 MHz, DMSO)  $\delta$  163.39, 154.53, 152.74, 151.97, 142.36, 139.75, 132.03, 131.77, 129.75, 127.24, 121.84, 119.79, 117.31, 111.98, 48.81, 21.73;  $[\text{M}+\text{H}]^+$  calculated for  $\text{C}_{17}\text{H}_{17}\text{N}_5\text{O}$  308.1, found 308.6.

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## EXAMPLE 13

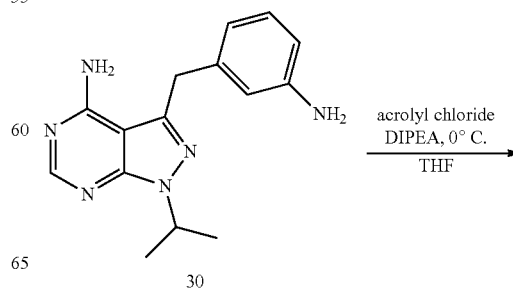
Preparation of N-(3-(1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-3-yl)phenyl)ethanesulfonamide (4)



Compound 4 was prepared by the same procedure that was used for compound 3 (18% yield):  $^1\text{H}$  NMR (400 MHz, DMSO)  $\delta$  10.17 (s, 1H), 9.61 (s, 1H), 9.08 (s, 1H), 7.90 (t,  $J=1.9$ , 1H), 7.82 (d,  $J=7.7$ , 1H), 7.49 (t,  $J=7.9$ , 1H), 7.29 (ddd,  $J=8.2$ , 2.2, 0.9, 1H), 6.87 (dd,  $J=16.4$ , 9.9, 1H), 6.17 (d,  $J=16.4$ , 1H), 6.07 (d,  $J=9.9$ , 1H), 5.23 (hept,  $J=6.7$ , 1H), 1.57 (d,  $J=6.7$ , 6H);  $^{13}\text{C}$  NMR (100 MHz, DMSO)  $\delta$  154.56, 152.69, 151.97, 141.98, 138.59, 136.20, 132.46, 130.16, 127.98, 122.09, 119.78, 117.64, 111.94, 48.89, 21.71;  $[\text{M}+\text{H}]^+$  calculated for  $\text{C}_{16}\text{H}_{17}\text{N}_5\text{O}_2\text{S}$  344.11, found 344.2.

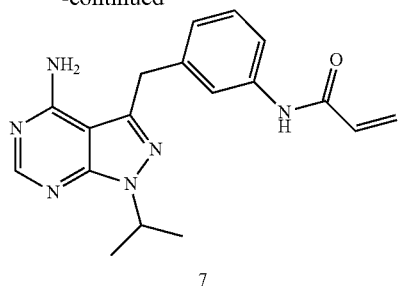
## EXAMPLE 14

Preparation of N-(3-((4-amino-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-3-yl)methyl)phenyl)acrylamide (7)



97

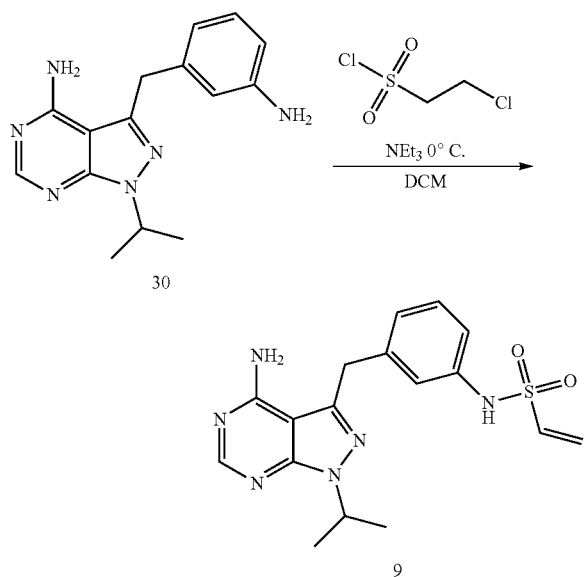
-continued



A solution of THF (20 mL), compound 30 (200 mg, 0.708 mmol) (prepared as in Dar et al. {Dar, 2008 #18}) and N,N-diisopropylethylamine (136  $\mu$ L, 0.781 mmol) was cooled 0° C., at which point freshly distilled acryloyl chloride (52  $\mu$ L, 0.642 mmol) was added. After one hour, the reaction mixture was concentrated in vacuo. The material was resuspended in dichloromethane (20 mL), which was washed with saturated sodium bicarbonate (20 mL). The aqueous layer was extracted with dichloromethane (3 $\times$ 20 mL) and the organic layers were subsequently combined, dried over MgSO<sub>4</sub>, filtered and concentrated to a solid. The material was purified by RP-HPLC and lyophilized to a white powder (113 mg, 47% yield): <sup>1</sup>H NMR (400 MHz, DMSO) 10.06 (s, 1H), 8.33 (s, 1H), 7.54 (s, 1H), 7.50 (d, J=8.1, 1H), 7.24 (t, J=7.9, 1H), 6.96 (d, J=7.7, 1H), 6.40 (dd, J=17.0, 10.1, 1H), 6.22 (dd, J=17.0, 2.0, 1H), 5.72 (dd, J=10.1, 1.9, 1H), 4.99 (hept, J=6.5, 1H), 4.40 (s, 2H), 1.46 (d, J=6.7, 6 H); <sup>13</sup>C NMR (100 MHz, DMSO)  $\delta$  163.06, 153.94, 151.30, 149.68, 144.69, 139.16, 139.08, 131.86, 128.82, 126.78, 123.59, 119.34, 117.51, 97.90, 48.74, 33.03, 21.70; [M+H]<sup>+</sup> calculated for C<sub>18</sub>H<sub>20</sub>N<sub>6</sub>O 337.2, found 337.4.

## EXAMPLE 15

Preparation of N-(3-((4-amino-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-3-yl)methyl)phenyl)ethene-sulfonamide (9)

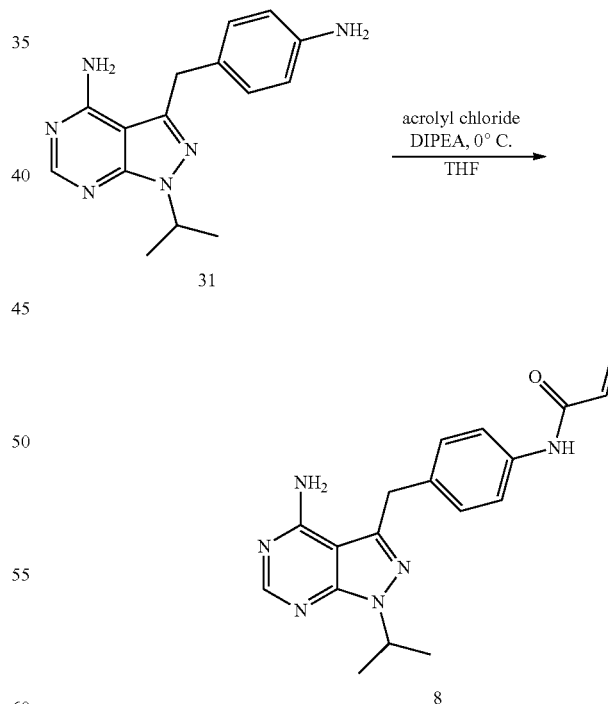


98

A solution of dichloromethane (20 mL), compound 30 (190 mg, 0.673 mmol) and triethylamine (188  $\mu$ L, 1.35 mmol) was cooled to 0° C. 2-chloro-1-ethane sulfonyl chloride (70  $\mu$ L, 0.670 mmol) was added and the reaction was allowed to proceed for 1 hour prior to addition of saturated sodium bicarbonate (20 mL) and extraction with dichloromethane (3 $\times$ 20 mL). The combined organic layers were dried with MgSO<sub>4</sub>, filtered and concentrated in vacuo. The product was purified by preparative RP-HPLC and lyophilized (14 mg, 6% yield): <sup>1</sup>H NMR (400 MHz, DMSO)  $\delta$  9.95 (s, 1H), 8.31 (s, 1H), 7.21 (t, J=8.1, 1H), 6.97 (m, 3H), 6.69 (dd, J=16.4, 9.9, 1H), 5.97 (d, J=16.5, 1H), 5.93 (d, J=9.9, 1H), 5.00 (hept, J=6.7, 1H), 4.38 (s, 2H), 1.46 (d, J=6.7, 6H); <sup>13</sup>C NMR (100 MHz, DMSO)  $\delta$  154.31, 151.46, 150.23, 144.31, 137.91, 136.26, 136.16, 129.25, 127.42, 123.79, 119.26, 117.52, 97.91, 48.59, 32.81, 21.70; [M+H]<sup>+</sup> calculated for C<sub>17</sub>H<sub>20</sub>N<sub>6</sub>O<sub>2</sub>S 373.1, found 373.4.

## EXAMPLE 16

Preparation of N-(4-((4-amino-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-3-yl)methyl)phenyl)acrylamide (8)



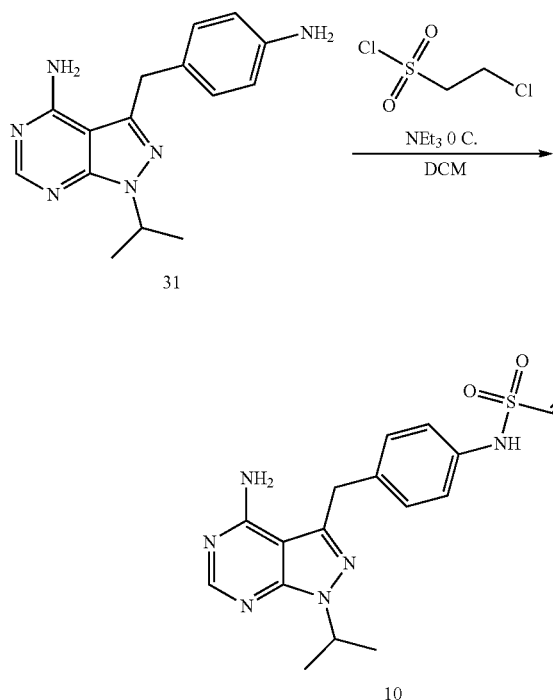
A solution of THF (20 mL), compound 31 (200 mg, 0.708 mmol) (prepared as in Dar et al. {Dar, 2008 #18}) and N,N-diisopropylethylamine (136  $\mu$ L, 0.781 mmol) was cooled 0° C., at which point freshly distilled acryloyl chloride (52  $\mu$ L,

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0.642 mmol) was added. After one hour, the reaction mixture was concentrated in vacuo. The material was resuspended in dichloromethane (20 mL), which was washed with saturated sodium bicarbonate (20 mL). The aqueous layer was extracted with dichloromethane (3×20 mL) and the organic layers were subsequently combined, dried over MgSO<sub>4</sub>, filtered and concentrated to a solid. The material was purified by RP-HPLC and lyophilized to a white powder (78 mg, 30% yield): <sup>1</sup>H NMR (400 MHz, DMSO) δ 10.09 (s, 1H), 8.31 (s, 1H), 7.57 (d, J=8.5, 2H), 7.18 (d, J=8.5, 2H), 6.41 (dd, J=17.0, 10.1, 1H), 6.22 (dd, J=17.0, 2.1, 1H), 5.73 (dd, J=10.1, 2.1, 1H), 5.00 (hept, J=6.7, 1H), 4.36 (s, 3H), 1.44 (d, J=6.7, 6H); <sup>13</sup>C NMR (100 MHz, DMSO) δ 163.0, 153.9, 151.3, 149.6, 145.1, 137.4, 133.4, 131.9, 128.7, 126.7, 119.5, 97.8, 48.7, 32.5, 21.6; [M+H]<sup>+</sup> calculated for C<sub>18</sub>H<sub>20</sub>N<sub>6</sub>O 337.2, found 337.4.

## EXAMPLE 17

Preparation of N-(4-((4-amino-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-3-yl)methyl)phenyl)ethanesulfonamide (10)



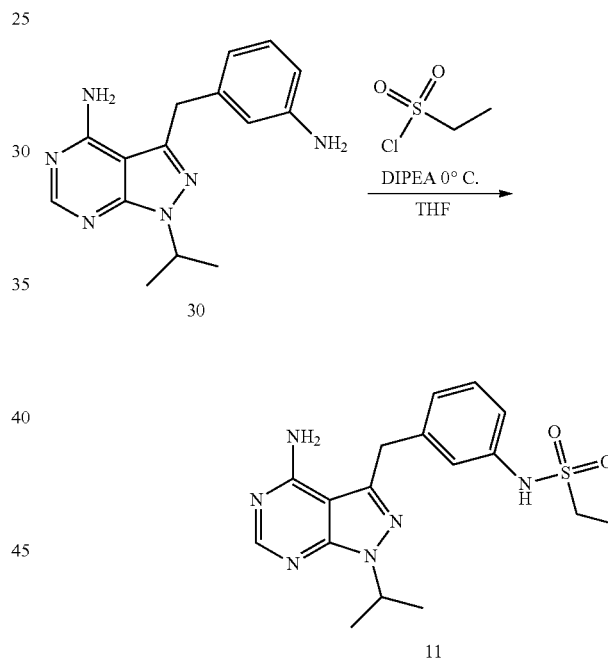
A solution of dichloromethane (20 mL), compound 31 (200 mg, 0.708 mmol) and triethylamine (200 μL, 1.43 mmol) was cooled to 0° C. 2-chloro-1-ethanesulfonyl chloride (70 μL, 0.670 mmol) was added and the reaction was allowed to proceed for 1 hour prior to addition of saturated sodium bicarbonate (20 mL) and extraction with dichloromethane (3×20 mL). The combined organic layers were

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dried with MgSO<sub>4</sub>, filtered and concentrated in vacuo. The product was purified by preparative RP-HPLC and lyophilized (14 mg, 6% yield): <sup>1</sup>H NMR (400 MHz, DMSO) δ 9.91 (s, 1H), 8.30 (s, 1H), 7.16 (d, J=8.3, 2H), 7.05 (d, J=8.4, 2H), 6.74 (dd, J=16.4, 9.9, 1H), 6.07 (d, J=16.4, 1H), 6.00 (d, J=10.0, 1H), 5.03-4.94 (m, 1H), 4.34 (s, 2H), 1.44 (d, J=6.6, 6H); <sup>13</sup>C NMR (100 MHz, DMSO) δ 154.0, 151.4, 149.8, 144.7, 136.3, 136.0, 133.9, 129.3, 127.5, 120.0, 97.9, 48.6, 32.3, 21.7; [M+H]<sup>+</sup> calculated for C<sub>17</sub>H<sub>20</sub>N<sub>6</sub>O<sub>2</sub>S 373.1, found 373.4.

## EXAMPLE 18

Preparation of N-(3-((4-amino-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-3-yl)methyl)phenyl)ethanesulfonamide (11)



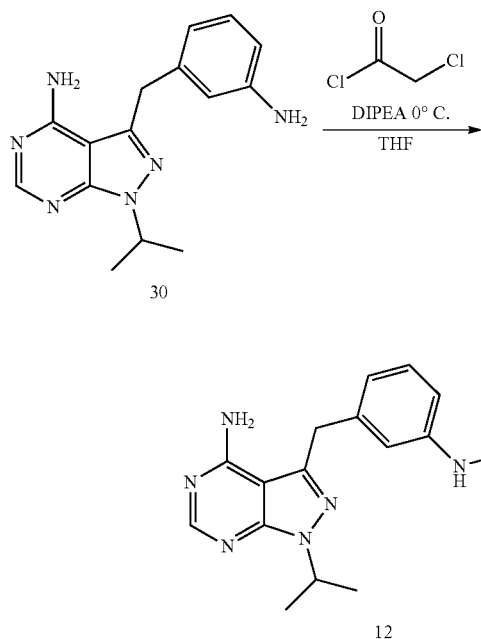
A solution of tetrahydrofuran (5 mL), compound 30 (50 mg, 0.177 mmol) and diisopropylethylamine (34 μL, 0.195 mmol) was cooled to 0° C. Ethanesulfonylchloride (15 μL, 0.159 mmol) was added and the reaction was allowed to proceed for one hour. After one hour, the reaction mixture was concentrated in vacuo. The material was resuspended in dichloromethane (10 mL), which was washed with saturated sodium bicarbonate (10 mL). The aqueous layer was extracted with dichloromethane (3×10 mL) and the organic layers were subsequently combined, dried over MgSO<sub>4</sub>, filtered and concentrated to a solid. The combined organic layers were dried with MgSO<sub>4</sub>, filtered and concentrated in vacuo. The product was purified by preparative RP-HPLC

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and lyophilized (25 mg, 38% yield):  $^1\text{H}$  NMR (400 MHz, DMSO)  $\delta$  9.72 (s, 1H), 8.34 (s, 1H), 7.23 (t,  $J=7.8$ , 1H), 7.09 (s, 1H), 7.03 (d,  $J=7.9$ , 1H), 6.98 (d,  $J=7.6$ , 1H), 5.01 (hept,  $J=6.7$ , 1H), 4.40 (s, 2H), 3.01 (q,  $J=7.3$ , 2H), 1.46 (d,  $J=6.7$ , 6H), 1.13 (t,  $J=7.3$ , 3H);  $^{13}\text{C}$  NMR (100 MHz, DMSO)  $\delta$  154.11, 151.38, 150.04, 144.45, 139.67, 138.54, 129.28, 123.66, 119.19, 117.45, 97.89, 48.57, 44.89, 32.84, 21.68, 7.90;  $[\text{M}+\text{H}]^+$  calculated for  $\text{C}_{17}\text{H}_{22}\text{N}_6\text{O}_2\text{S}$  375.1, found 375.6.

## EXAMPLE 19

Preparation of N-(3-((4-amino-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-3-yl)methyl)phenyl)-2-chloroacetamide (12)



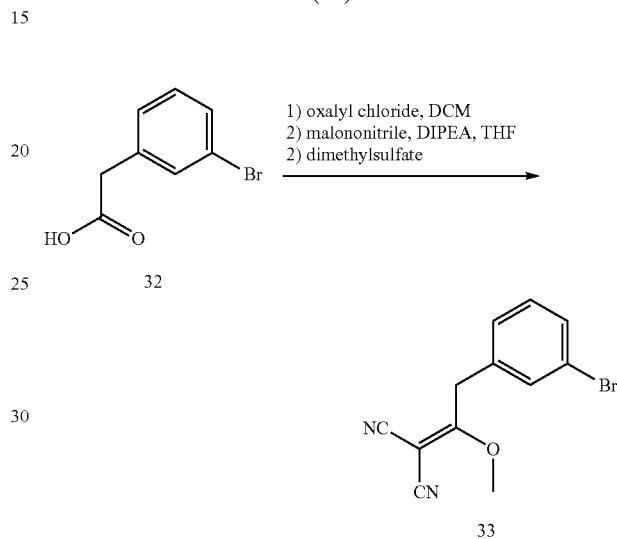
A solution of tetrahydrofuran (10 mL), compound 30 (100 mg, 0.354 mmol) and diisopropylethylamine (68  $\mu\text{L}$ , 0.390 mmol) was cooled to  $0^\circ\text{C}$ . Chloroacetyl chloride (25.4  $\mu\text{L}$ , 0.313 mmol) was added and the reaction was allowed to proceed for one hour. After one hour, the reaction mixture was concentrated in vacuo. The material was resuspended in dichloromethane (10 mL), which was washed with saturated sodium bicarbonate (10 mL). The aqueous layer was extracted with dichloromethane (3 $\times$ 10 mL) and the organic layers were subsequently combined, dried over  $\text{MgSO}_4$ , filtered and concentrated to a solid. The combined organic layers were dried with  $\text{MgSO}_4$ , filtered and concentrated in vacuo. The product was purified by preparative RP-HPLC and lyophilized (35 mg, 31% yield):  $^1\text{H}$  NMR (400 MHz, DMSO)  $\delta$  10.22 (s, 1H), 8.32 (s, 1H), 7.46 (s, 1H), 7.41 (d,  $J=8.2$ , 1H), 7.25 (t,  $J=7.8$ , 1H), 6.99 (d,  $J=7.6$ , 1H), 5.00 (hept,  $J=6.7$ , 1H), 4.40 (s, 2H), 4.20 (s, 2H), 1.46 (d,  $J=6.7$ ,

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6H);  $^{13}\text{C}$  NMR (100 MHz, DMSO)  $\delta$  164.5, 154.0, 151.3, 150.0, 144.6, 139.2, 138.6, 128.9, 124.0, 119.3, 117.5, 97.9, 48.7, 43.6, 33.0, 21.7;  $[\text{M}+\text{H}]^+$  calculated for  $\text{C}_{17}\text{H}_{19}\text{ClN}_6\text{O}$  359.1, found 359.2.

## EXAMPLE 20

Preparation of  
2-(methoxy(3-bromobenzyl)methylene)malononitrile  
(33)

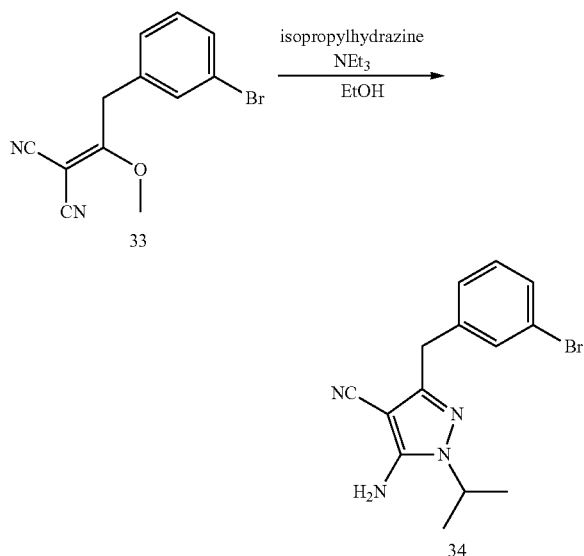


A solution of 3-bromoacetyl acetic acid (5 g, 23.3 mmol) was mixed with oxalyl chloride (10 mL, 121 mmol) in dichloromethane (75 mL) at room temperature for 30 minutes and then concentrated in vacuo. To the resulting solid was added malononitrile (1.69 g, 25.6 mmol) and THF (25 mL). After cooling to  $0^\circ\text{C}$ , DIPEA (10.1 mL, 58.1 mmol) was added dropwise and the reaction was allowed to warm to room temperature and proceed for 2 hours. Afterwards, dimethylsulfate (6.60 mL, 69.3 mmol) was added and the temperature was raised to  $70^\circ\text{C}$  for 4 hours. Next, the reaction mixture was brought to room temperature and allowed to proceed for an additional 12 hours. EtOAc (50 mL) was added to the reaction mixture in addition to brine (50 mL). The organic and aqueous layers were separated and the aqueous layer was extracted with EtOAc (4 $\times$ 25 mL). The combined organic layers were dried with  $\text{MgSO}_4$  and concentrated in vacuo. The material was purified over a silica column using chloroform:hexane (90:10) initially and eluted with pure chloroform. After concentrating the fractions containing the product, an amber oil was triturated with diethyl ether to yield a solid (2.057 g, 32% yield):  $^1\text{H}$  NMR (400 MHz, DMSO)  $\delta$  7.56 (m, 2H), 7.38 (t,  $J=7.7$ , 1H), 7.31 (d,  $J=7.7$ , 1H), 4.19 (s, 2H), 4.01 (s, 3H);  $^{13}\text{C}$  NMR (100 MHz, DMSO)  $\delta$  187.96, 135.22, 131.33, 131.18, 130.77, 127.18, 122.32, 113.75, 112.03, 65.62, 60.00, 35.60;  $[\text{M}+\text{H}]^+$  calculated for  $\text{C}_{12}\text{H}_9\text{BrN}_2\text{O}$  274.9, 276.9 (50:50), found 274.8, 276.5.

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## EXAMPLE 21

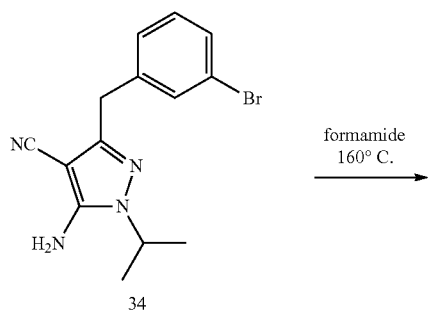
Preparation of 5-amino-3-(3-bromobenzyl)-1-isopropyl-1H-pyrazole-4-carbonitrile (34)



Compound 33 (3.124 g, 11.3 mmol), isopropylhydrazine hydrochloride (1.27 g, 11.5 mmol) (purchased from Ryan Scientific) and triethylamine (6.40 mL, 46.0 mmol) were allowed to react in ethanol (75 mL) at room temperature for 1 hr. After concentrating the reaction mixture, it was purified by silica chromatography using a chloroform/methanol solvent system (methanol gradient increased with time from 0-10%). The relevant fractions were concentrated in vacuo to yield a yellow powder (3.15 g, 87% yield):  $^1\text{H}$  NMR (400 MHz, DMSO)  $\delta$  7.41 (m, 2H), 7.26 (td,  $J=7.7$ , 1.2, 1H), 7.21 (dt,  $J=7.7$ , 1.3, 1H), 6.50 (s, 2H), 4.38 (hept,  $J=6.5$ , 1H), 3.81 (s, 2H), 1.27 (d,  $J=6.5$ , 6H);  $^{13}\text{C}$  NMR (100 MHz, DMSO)  $\delta$  151.01, 150.15, 141.24, 131.05, 130.53, 129.16, 127.45, 121.55, 115.16, 71.77, 47.24, 32.88, 21.35;  $[\text{M}+\text{H}]^+$  calculated for  $\text{C}_{14}\text{H}_{15}\text{BrN}_4$  319.0, 321.0 found 318.9: 321.0.

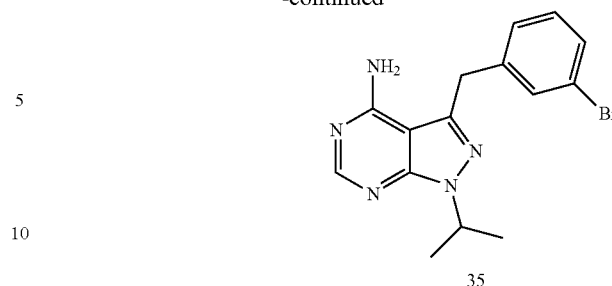
## EXAMPLE 22

Preparation of 3-(3-bromobenzyl)-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine



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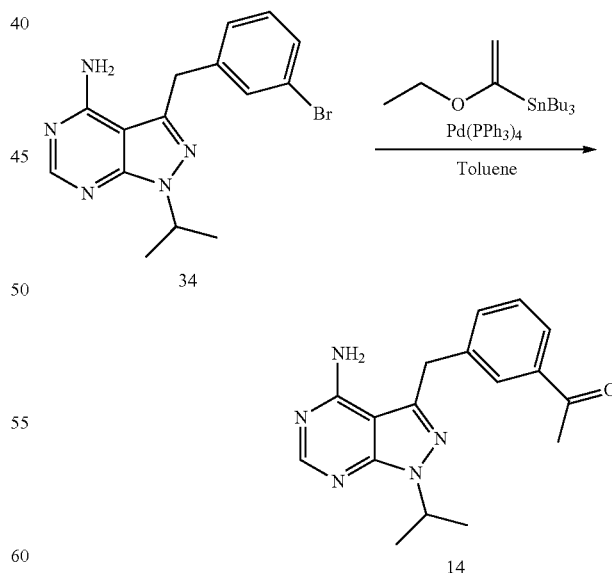
## -continued



Compound 34 (6.09 g, 19.1 mmol) was added to formamide (26.6 mL, 668 mmol) and heated to 160°C. for 27 hours. Afterwards, the reaction mixture was allowed to cool to room temperature and diluted into ice cold water (50 mL). A viscous material was filtered and dissolved in EtOAc. This solution was washed with brine and concentrated in vacuo (6.23 g, 91% yield):  $^1\text{H}$  NMR (400 MHz, DMSO)  $\delta$  8.13 (s, 1H), 7.49 (s, 1H), 7.37 (dt,  $J=7.5$ , 1.7, 1H), 7.22 (m, 2H), 4.95 (hept,  $J=6.6$ , 1H), 4.38 (s, 2H), 1.43 (d,  $J=6.7$ , 6H);  $^{13}\text{C}$  NMR (100 MHz, DMSO)  $\delta$  157.90, 155.39, 153.22, 142.01, 141.86, 131.16, 130.52, 129.00, 127.40, 121.56, 98.39, 47.76, 32.66, 21.71;  $[\text{M}+\text{H}]^+$  calculated for  $\text{C}_{15}\text{H}_{16}\text{BrN}_5$  346.0, 348.0 found 346.0: 348.0.

## EXAMPLE 23

Preparation of 3-(3-acetylbenzyl)-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine (14)



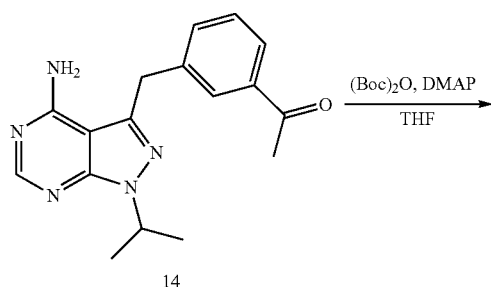
Anhydrous toluene (10 mL) was degassed prior to addition of 34 (3.5 g, 10.1 mmol) Tributyl(1-ethoxyvinyl)tin (4.081 mL, 12.1 mmol), tetrakis(triphenylphosphine) palladium (1.169 g, 10 mol %) and heating to 120°C. After 16 hours, the reaction mixture was concentrated in vacuo. Next, a THF/1M

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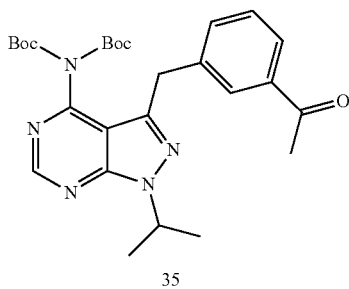
HCl solution (33 mL/10 mL) was added to the brownish material and the reaction was allowed to proceed at room temperature for 12 hours. Afterwards, EtOAc (175 mL) was added to the mixture, which was washed with saturated sodium bicarbonate (700 mL) and extracted with 1 M HCl (2×525 mL). The pH was adjusted to a value of 13 and the mixture was extracted with EtOAc (2×525 mL). The organic layers were dried with sodium sulfate and concentrated in vacuo. The material was purified by silica chromatography using a chloroform/methanol solvent system (methanol gradient increased with time from 0-8%). The relevant fractions were concentrated in vacuo to yield a solid (2.035 g, 65% yield): <sup>1</sup>H NMR (400 MHz, DMSO) δ 8.13 (s, 1H), 7.91 (s, 1H), 7.79 (d, J=7.5, 1H), 7.48 (d, J=7.7, 1H), 7.43 (t, J=7.6, 1H), 4.97 (hept, J=6.7, 1H), 4.46 (s, 2H), 2.53 (s, 3H), 1.44 (d, J=6.7, 6H); <sup>13</sup>C NMR (100 MHz, DMSO) δ 197.80, 157.96, 155.40, 153.24, 142.22, 139.80, 136.84, 133.15, 128.75, 128.10, 126.32, 98.40, 47.71, 32.94, 26.70, 21.77; [M+H]<sup>+</sup> calculated for C<sub>17</sub>H<sub>19</sub>N<sub>5</sub>O 310.1, found 310.0.

## EXAMPLE 24

Preparation of 3-(3-acetylbenzyl)-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-4-di-*t*-butoxycarbonyl amine (14)



14



35

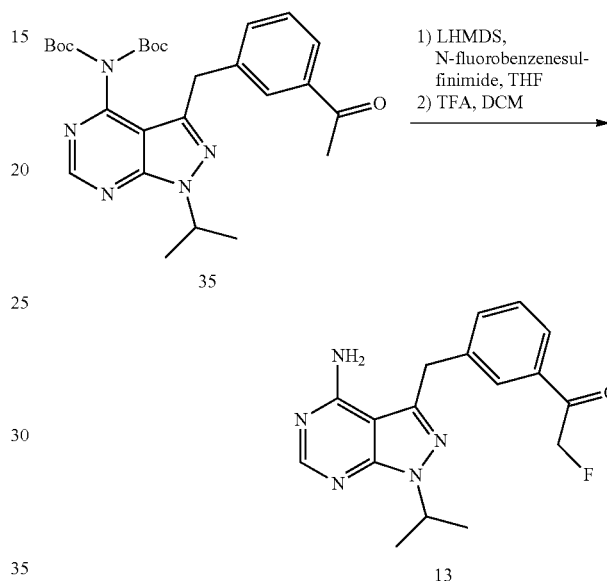
Compound 14, di-*tert*-butyldicarbonate and dimethylaminopyridine were mixed and allowed to react for 3 hours at room temperature. Afterwards, the reaction mixture was diluted with EtOAc and washed with 1 M HCl and brine. The organic solution was dried with MgSO<sub>4</sub> and concentrated in vacuo. The material was purified over a silica column using a hexane/ethyl acetate solvent system (1.834 g, 55% yield): <sup>1</sup>H NMR (400 MHz, DMSO) δ 8.92 (s, 1H), 7.81 (d, J=7.9, 1H), 7.77 (s, 1H), 7.41 (t, J=7.7, 1H), 7.29 (d, J=7.7, 1H), 5.20 (hept, J=6.7, 1H), 4.25 (s, 2H), 2.53 (s, 3H), 1.55 (d, J=6.7, 6H), 1.28 (s, 18H). <sup>13</sup>C NMR (100 MHz, DMSO) δ 197.42, 154.90, 154.28, 153.19, 149.83, 141.77, 138.25, 136.88,

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132.65, 128.85, 127.74, 126.52, 109.01, 83.67, 49.08, 33.51, 27.19, 26.58, 21.63; [M+H]<sup>+</sup> calculated for C<sub>27</sub>H<sub>35</sub>N<sub>5</sub>O<sub>5</sub> 510.2, found 510.1.

## EXAMPLE 25

Preparation of 1-(3-((4-amino-1-isopropyl-1H-pyrazolo[3,4-d]pyrimidin-3-yl)methyl)phenyl)-2-fluoroethanone (13)



1) LHMDS,  
N-fluorobenzenesulfinamide, THF  
2) TFA, DCM

13

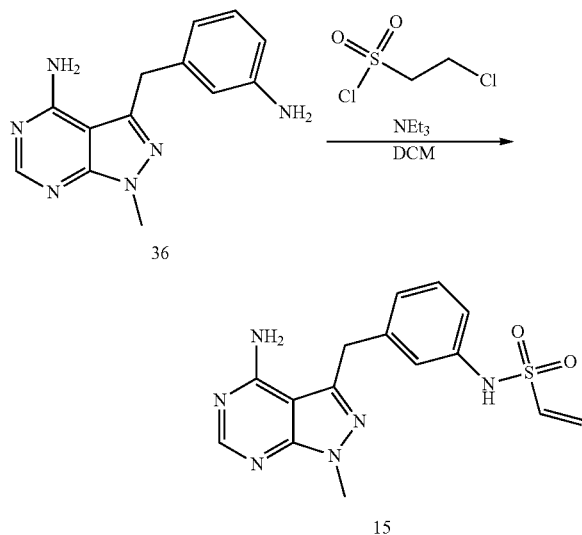
Compound 35 (253 mg, 0.496 mmol) was mixed with anhydrous THF (2 mL) and cooled to -78° C., whereupon 1.0 M LHMDS in THF (0.645 mL, 0.645 mmol) was added dropwise via syringe and allowed to react for 15 minutes. N-fluorobenzenesulfinamide (250 mg, 0.794 mmol) in THF (2 mL) was then added dropwise and the reaction mixture was allowed to come to room temperature over 30 minutes. The reaction mixture was cooled to -78° C. and saturated ammonium chloride (100 mL) was added dropwise. The reaction mixture was extracted with EtOAc (70 mL), and the resulting organic layer was washed with saturated sodium bicarbonate (1×70 mL) and brine (1×70 mL). The organic layer was concentrated in vacuo to give a yellow oil. The resulting product was purified over a silica column (hexane/ethyl acetate solvent system) and fractions containing the monofluorinated product and unreacted material (35) were pooled (they were inseparable). This combined mixture (93 mg) was reacted with TFA (1.5 mL) in DCM for 5 hours at room temperature and then concentrated in vacuo. The Boc-protected material was then resuspended in EtOAc (30 mL) and washed with saturated sodium bicarbonate (1×30 mL) and brine (1×30 mL). After concentrating the organic layer, the monofluorinated product was purified by preparative TLC using a 8% MeOH/CHCl<sub>3</sub> solvent system (22 mg, 14% yield): <sup>1</sup>H NMR (400 MHz, DMSO) δ 9.91 (s, 1H), 8.30 (s, 1H), 7.16 (d, J=8.3, 2H), 7.05 (d, J=8.4, 2H), 6.74 (dd, J=16.4, 9.9, 1H), 6.07 (d, J=16.4, 1H), 6.00 (d, J=10.0, 1H), 5.03-4.94 (m, 1H), 4.34 (s, 2H), 1.44 (d, J=6.6, 6H); <sup>13</sup>C NMR (100 MHz, DMSO) δ 154.0, 151.4, 149.8, 144.7, 136.3, 136.0, 133.9,

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129.3, 127.5, 120.0, 97.9, 48.6, 32.3, 21.7;  $[M+H]^+$  calculated for  $C_{17}H_{18}FN_5O$  328.1, found 328.1.

## EXAMPLE 26

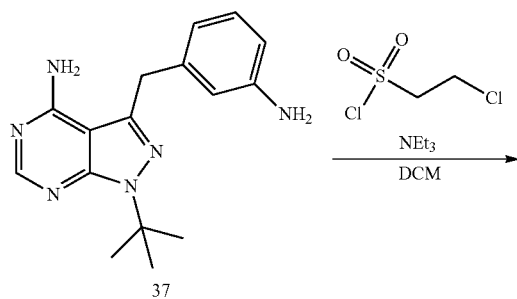
Preparation of N-(3-((4-amino-1-methyl-1H-pyrazolo[3,4-d]pyrimidin-3-yl)methyl)phenyl)ethanesulfonamide (15)



Compound 36 was prepared by the method of Dar et al. {Dar, 2008 #18}. Compound 15 was prepared by the same method used for compound 9 (22% yield): <sup>1</sup>H NMR (400 MHz, DMSO) δ 9.94 (s, 1H), 8.36 (s, 1H), 7.21 (t, J=7.8, 1H), 6.98 (m, 3H), 6.71 (dd, J=16.4, 9.9, 1H), 6.03 (d, J=16.4, 1H), 5.97 (d, J=9.9, 1H), 4.36 (s, 2H), 3.91 (s, 3H); <sup>13</sup>C NMR (100 MHz, DMSO) δ 154.2, 152.3, 150.4, 144.6, 139.4, 137.9, 136.2, 129.2, 127.6, 124.0, 119.4, 117.5, 97.8, 33.7, 32.8;  $[M+H]^+$  calculated for  $C_{15}H_{16}N_6O_2S$  345.1, found 345.4.

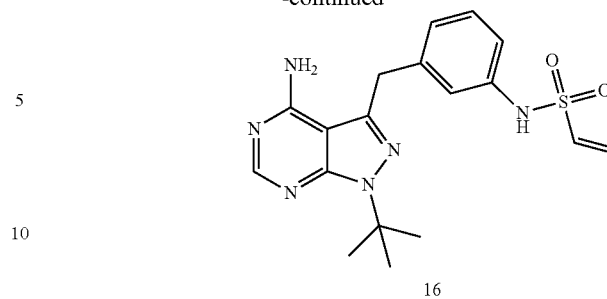
## EXAMPLE 27

Preparation of N-(3-((4-amino-1-tert-butyl-1H-pyrazolo[3,4-d]pyrimidin-3-yl)methyl)phenyl)ethanesulfonamide (16)



## 108

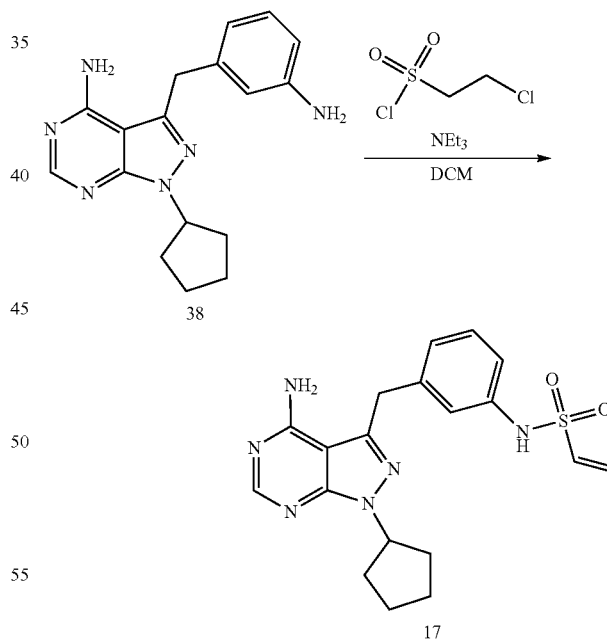
-continued



Compound 37 was prepared by the method of Dar et al. {Dar, 2008 #18}. Compound 16 was prepared by the same method used for compound 9 (10% yield): <sup>1</sup>H NMR (400 MHz, DMSO) δ 9.95 (s, 1H), 8.30 (s, 1H), 7.21 (t, J=8.1, 1H), 6.97 (m, 3H), 6.69 (dd, J=16.4, 9.9, 1H), 5.94 (m, 2H), 4.36 (s, 2H), 1.71 (s, 9H); <sup>13</sup>C NMR (100 MHz, DMSO) δ 154.5, 152.3, 149.6, 142.5, 139.6, 137.9, 136.2, 129.1, 127.4, 123.8, 119.1, 117.4, 99.1, 60.2, 32.8, 28.8;  $[M+H]^+$  calculated for  $C_{18}H_{22}N_6O_2S$  387.2, found 387.5.

## EXAMPLE 28

Preparation of N-(3-((4-amino-1-cyclopentyl-1H-pyrazolo[3,4-d]pyrimidin-3-yl)methyl)phenyl)ethanesulfonamide (17)



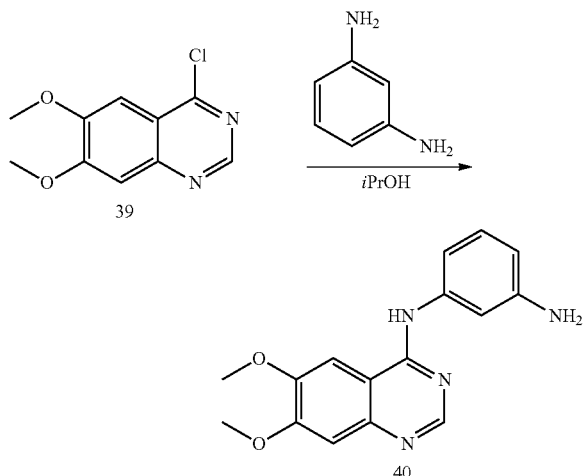
Compound 38 was prepared by the method of Dar et al. {Dar, 2008 #18}. Compound 17 was prepared by the same method used for compound 9 (18% yield): <sup>1</sup>H NMR (400 MHz, DMSO) δ 9.95 (s, 1H), 8.37 (d, J=6.8, 1H), 7.21 (t, J=8.0, 1H), 6.98 (m, 3H), 6.69 (dd, J=16.4, 9.9, 1H), 5.98 (d, J=16.4, 1H), 5.93 (d, J=9.9, 1H), 5.18 (t, J=7.2, 1H), 4.38 (s, 2H), 2.08 (m, 2H), 1.98 (m, 2H), 1.88 (m, 2H), 1.68 (m, 2H); <sup>13</sup>C NMR (100 MHz, DMSO) δ 153.7, 151.7, 149.6, 144.7,

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139.5, 137.9, 136.2, 129.1, 127.4, 123.8, 119.2, 117.5, 97.9, 57.2, 32.8, 31.9, 24.3; [M+H]<sup>+</sup> calculated for C<sub>19</sub>H<sub>22</sub>N<sub>6</sub>O<sub>2</sub>S 399.2, found 399.4.

## EXAMPLE 29

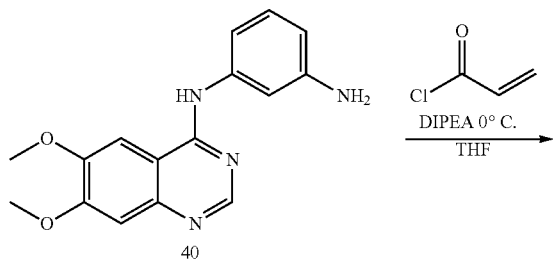
Preparation of N1-(6,7-dimethoxyquinazolin-4-yl) benzene-1,3-diamine



Compound 39 was prepared by a previously described method {Perera, 2008 #110}. Compound 39 (300 mg, 1.34 mmol) and 1,3-phenylenediamine (1.78 g, 16.5 mmol) were heated to 90° C. in isopropanol and allowed to react for 1.5 hours, after which the reaction was brought to room temperature. The resulting green, solid product was collected by filtration and washed with cold isopropanol (173 mg, 44% yield): <sup>1</sup>H NMR (400 MHz, DMSO) δ 11.01 (br, 1H), 8.72 (s, 1H), 8.24 (s, 1H), 7.36 (s, 1H), 7.15 (t, J=8.0, 1H), 7.00 (s, 1H), 6.93 (d, J=7.9, 1H), 6.62 (d, J=8.0, 1H), 3.99 (s, 3H), 3.97 (s, 3H); <sup>13</sup>C NMR (100 MHz, DMSO) δ 157.85, 155.85, 149.92, 149.21, 137.73, 129.08, 113.52, 112.92, 110.99, 107.35, 103.60, 100.86, 56.73, 56.33; [M+H]<sup>+</sup> calculated for C<sub>16</sub>H<sub>16</sub>N<sub>4</sub>O<sub>2</sub> 297.1, found 297.4.

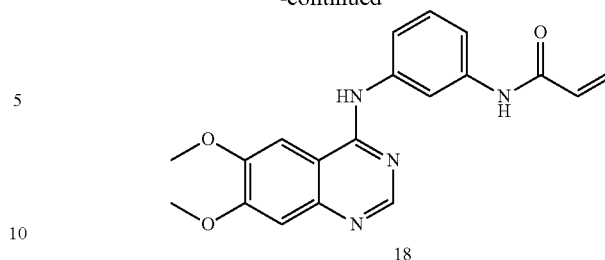
## EXAMPLE 30

Preparation of N-(3-(6,7-dimethoxyquinazolin-4-ylamino)phenyl)acrylamide (18)



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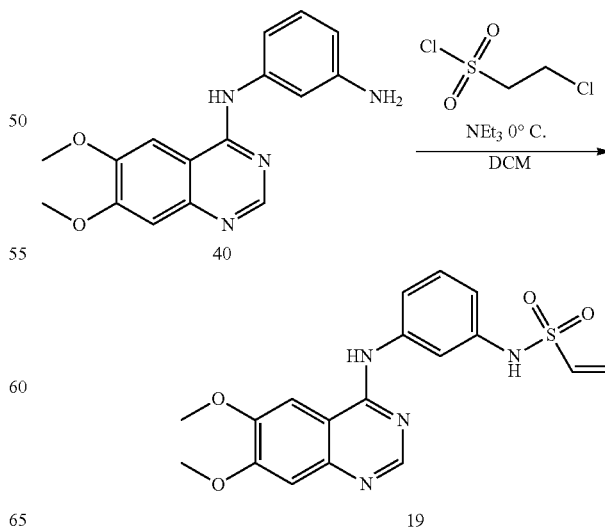
-continued



A solution of THF (7 mL), compound 40 (75 mg, 0.253 mmol) and 1*N,N*-diisopropylethylamine (96 μL, 0.551 mmol) was cooled 0° C., at which point freshly distilled acryloyl chloride (19 μL, 0.230 mmol) was added. After one hour, the reaction mixture was concentrated in vacuo. The material was resuspended in dichloromethane (10 mL), which was washed with saturated sodium bicarbonate (10 mL). The aqueous layer was extracted with dichloromethane (2×10 mL) and the organic layers were subsequently combined, dried over MgSO<sub>4</sub>, filtered and concentrated to a solid. The material was purified by RP-HPLC and lyophilized to a powder (40 mg, 50% yield): <sup>1</sup>H NMR (400 MHz, DMSO) δ 10.81 (br, 1H), 10.31 (s, 1H), 8.78 (s, 1H), 8.17 (s, 1H), 8.06 (s, 1H), 7.41 (m, 3H), 7.25 (s, 1H), 6.47 (dd, J=17.0, 10.1, 1H), 6.28 (dd, J=17.0, 1.9, 1H), 5.79 (dd, J=10.1, 1.9, 1H), 4.00 (s, 6H); <sup>13</sup>C NMR (100 MHz, DMSO) δ 163.32, 158.05, 156.18, 150.11, 149.32, 139.48, 137.32, 131.73, 129.02, 127.18, 119.88, 117.10, 115.49, 107.30, 103.23, 100.68, 56.57, 56.45; [M+H]<sup>+</sup> calculated for C<sub>19</sub>H<sub>18</sub>N<sub>4</sub>O<sub>3</sub> 351.1, found 351.4.

## EXAMPLE 31

Preparation of N-(3-(6,7-dimethoxyquinazolin-4-ylamino)phenyl)ethanesulfonamide (19)

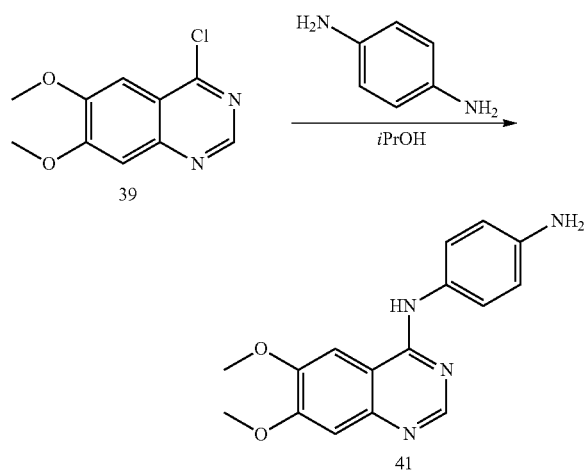


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A solution of dichloromethane (10 mL), compound 40 (97 mg, 0.327 mmol) and triethylamine (138  $\mu$ L, 0.989 mmol) was cooled to 0° C. 2-chloro-1-ethane sulfonyl chloride (30  $\mu$ L, 0.287 mmol) was added and the reaction was allowed to proceed for 1 hour prior to removal of a greenish precipitate and addition of saturated sodium bicarbonate (10 mL) and extraction with dichloromethane (2 $\times$ 10 mL). The combined organic layers were dried with MgSO<sub>4</sub>, filtered and concentrated in vacuo. The product (organic layers and green precipitate combined) was purified by preparative RP-HPLC and lyophilized (40 mg, 36% yield): <sup>1</sup>H NMR (400 MHz, DMSO)  $\delta$  10.71 (br, 1H), 10.20 (s, 1H), 8.76 (s, 1H), 8.02 (s, 1H), 7.52 (s, 1H), 7.39 (m, 2H), 7.25 (s, 1H), 7.06 (m, 1H), 6.82 (dd, J=16.4, 9.9, 1H), 6.18 (d, J=16.4, 1H), 6.10 (d, J=9.9, 1H), 3.99 (s, 6H); <sup>13</sup>C NMR (100 MHz, DMSO)  $\delta$  157.94, 156.12, 150.06, 149.43, 138.29, 137.85, 136.06, 129.40, 128.11, 119.92, 117.13, 115.32, 107.41, 103.15, 100.96, 56.55, 56.42; [M+H]<sup>+</sup> calculated for C<sub>18</sub>H<sub>18</sub>N<sub>4</sub>O<sub>4</sub>S 387.1, found 387.5.

## EXAMPLE 32

Preparation of N1-(6,7-dimethoxyquinazolin-4-yl) benzene-1,4-diamine (41)



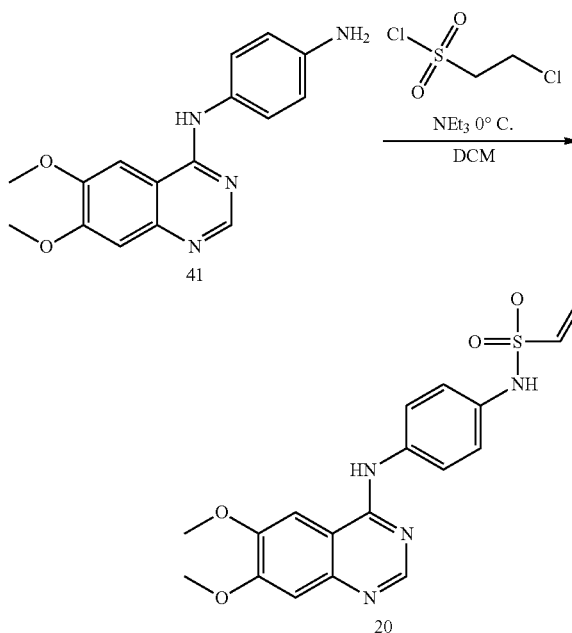
Compound 39 was prepared by a previously described method {Perera, 2008 #110}. Compound 39 (600 mg, 2.68 mmol) and 1,3-phenylenediamine (3.57 g, 33 mmol) were heated to 90° C. in isopropanol and allowed to react for 1.5 hours, after which the reaction was brought to room temperature. The resulting solid product was collected by filtration and washed with cold isopropanol (775 mg, 98% yield): <sup>1</sup>H NMR (400 MHz, DMSO)  $\delta$  10.38 (br, 1H), 8.59 (s, 1H), 8.00 (s, 1H), 7.28 (d, J=8.6, 2H), 7.20 (s, 1H), 6.79 (s, 1H), 6.66 (d, J=8.6, 2H), 3.96 (s, 6H); <sup>13</sup>C NMR (100 MHz, DMSO)  $\delta$  157.31, 155.07, 150.47, 149.39, 145.88, 126.53, 125.38,

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113.97, 107.62, 103.05, 102.79, 56.49, 56.10; [M+H]<sup>+</sup> calculated for C<sub>16</sub>H<sub>16</sub>N<sub>4</sub>O<sub>2</sub> 297.1, found 297.4.

## EXAMPLE 33

Preparation of N-(4-(6,7-dimethoxyquinazolin-4-ylamino)phenyl)ethanesulfonamide (20)



A solution of dichloromethane (10 mL), compound 41 (100 mg, 0.337 mmol) and triethylamine (141  $\mu$ L, 1.02 mmol) was cooled to 0° C. 2-chloro-1-ethane sulfonyl chloride (32  $\mu$ L, 0.304 mmol) was added and the reaction was allowed to proceed for 1 hour prior to removal of a precipitate and addition of saturated sodium bicarbonate (10 mL) and extraction with dichloromethane (2 $\times$ 10 mL). The combined organic layers were dried with MgSO<sub>4</sub>, filtered and concentrated in vacuo. The product was purified by preparative RP-HPLC and lyophilized (24 mg, 20% yield): <sup>1</sup>H NMR (400 MHz, DMSO)  $\delta$  10.81 (br, 1H), 10.13 (s, 1H), 8.75 (s, 1H), 8.02 (s, 1H), 7.58 (d, J=8.9, 2H), 7.25 (m, 3H), 6.83 (dd, J=16.4, 10.0, 1H), 6.15 (d, J=16.5, 1H), 6.07 (d, J=10.0, 1H), 3.99 (s, 3H), 3.98 (s, 3H); <sup>13</sup>C NMR (100 MHz, DMSO)  $\delta$  157.93, 156.16, 150.10, 149.26, 136.23, 135.75, 132.76, 127.82, 125.54, 120.00, 107.16, 103.16, 100.51, 56.55, 56.45; [M+H]<sup>+</sup> calculated for C<sub>18</sub>H<sub>18</sub>N<sub>4</sub>O<sub>4</sub>S 387.1, found 387.4.

## EXAMPLE 34

## Gel Filtration and Kinetic Assays

Inhibitors (final concentration 23  $\mu$ M) were incubated with c-Src variants (final concentration 11.5  $\mu$ M) in kinase reaction buffer (50 mM Tris pH 8, 100 mM NaCl, 1 mM DTT, 5% glycerol, 5% DMSO) for 25 minutes at room temperature. The solutions (2.6 mL total) were then passed over PD10 desalting columns (GE Healthcare) using the kinase reaction buffer for elution. Src concentrations were calculated using the extinction coefficient for c-Src (51,140 mM<sup>-1</sup>cm<sup>-1</sup>)

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{Seeliger, 2005 #100}. Kinase assays were performed as described in the experimental section at a final enzyme concentration of 10 nM.

Protein labeling conditions: Kinase labeling reactions were performed by incubating 30  $\mu$ L quantities of c-Src variants (in 50 mM Tris pH 8, 100 mM NaCl, 1 mM DTT, 5% glycerol) with two equivalents of inhibitor in DMSO (final DMSO concentration=2.4%). The covalent labeling reaction was quenched by removing 4  $\mu$ L and adding it to 31  $\mu$ L of 0.1% formic acid. The sample was then analyzed by ES1-oe-TOF mass spectrometry.

## EXAMPLE 35

## Crystallization and Data Collection for c-Src-ES-9

The c-Src-ES variant was prepared and purified as described above, run over a 0.22  $\mu$ m PVDF centrifugal filter and diluted to 1.5-3 mg/ml in 50 mM Tris (pH 8.0), 100 mM NaCl, 5% (v/v) glycerol, 1 mM DTT. Compound 9 was freshly dissolved in DMSO and added to the protein solutions (1.5-3 equivalents). After 1.5 hours of incubation at room temperature, the reaction mixtures were spun at 10,000 rpm and the supernatants were collected. Hanging drop crystallization conditions were set up by mixing 1:1 protein and precipitation solutions (100 mM MES (pH 6.5), 50 mM NaOAc, 4-8% PEG 4000). After 24-48 hours at room temperature, thin plate-like crystals were observed. Crystals were cryoprotected in the crystallization solution supplemented with 25% glycerol and stored in liquid nitrogen prior to obtaining diffraction data at beamline 8.2.2 (wavelength of 1.0088 nm, nitrogen gas stream at 100 K) at the Berkeley Lab Advanced Light Source. Data was processed with HKL2000 (HKL Research, Inc.) and Phenix software {Adams, #108}.

Crystal structure of c-Src-ES1 with 9—In order to elucidate the binding mode for a kinase with a cysteine gatekeeper and an irreversible inhibitor, an X-ray crystal structure of the catalytic domain of c-Src-ES1 (residues 251-533) bound to 9 was solved (FIG. 2). Co-crystallization through incubation of c-Src-ES1 with 9 was performed using hanging-drop vapor diffusion. The complex was solved by molecular replacement and, contained two molecules in the crystallographic asymmetric unit of the P1 space group. The structure was refined to 2.2 Å and exhibited electron density for 9 covalently bound to Cys338. Poor electron density was observed near the N-terminus (residues 251-256) and in flexible regions of the kinase such as the glycine-rich loop (residues 275-278) and the activation segment (residues 407-424). However, the DFG motif at the beginning of the activation segment (residues 404-406) was clearly resolved and was in the conformation associated with an active kinase (DFG-in).

The binding mode of 9 (a vinylsulfonamide functionalized compound) with c-Src-ES1 is related to Type I $\frac{1}{2}$  kinase inhibition. Like Type I inhibitors, Type I $\frac{1}{2}$  inhibitors bind the active conformation of the kinase (DFG-in) and engage in a series of hydrogen bonds in the hinge region. Type I $\frac{1}{2}$  are similar to Type II inhibitors in that they occupy the pocket situated behind the gatekeeper and hydrogen bond to the carboxylate of the conserved glutamate on the  $\alpha$ C-helix and backbone amide of the DFG aspartate (FIG. 2C). The hydrogen bonds afforded by the tetrahedral arrangement of the sulfone may contribute to the increased potency of 9 relative to 7, which contains an acrylamide.

## EXAMPLE 36

## Kinome-Wide Profiling of Inhibitors

The percent inhibition results in FIG. were generated with biochemical enzymatic kinase assays using the

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SelectScreen® Kinase Profiling Service (Life Technologies Corporation, Madison, Wis.). Compounds were assayed at 1  $\mu$ M at an ATP concentration equal to the ATP  $K_m$  app for the assay following the detailed procedures described in the SelectScreen® Customer Protocol and Assay Conditions documents located at [www.invitrogen.com/kinaseprofiling](http://www.invitrogen.com/kinaseprofiling).

In order to identify potential off-targets, a panel of the electrophilic inhibitors that showed inhibition of c-Src-ES1 against 307 kinases was screened (Table 5). Compounds that were profiled include, but are not limited to, 3, 4, 9, 13, and 20. Excluding 3, all of the compounds had relatively few off-target effects. The exocyclic amine mimics N6 of ATP and plays an important role in a hydrogen bonding interaction with the hinge region of kinases. Several of the kinases for which >80% inhibition was achieved with vinylsulfonamide-based inhibitors was observed are those with exposed cysteines near the active site (e.g. EGFR, HER4, BTK, BMX, TXK). The fluoromethylketone-type compound, 13, had a clean profile against kinases in the panel. The present invention provides one of the most selective chemical genetic kinase inhibitor reported to date.

## EXAMPLE 37

## Site Directed Mutagenesis

The T338C mutation was introduced to a pET-28 vector containing a hexahistidine-tagged Src construct using standard site directed mutagenesis methods. The protein was produced in *E. coli* BL21DE3 cells containing YopH phosphatase and GroEL. The cells were grown in Terrific Broth containing (kanamycin, 50 mg/mL/streptomycin, 50 mg/mL). Cells were grown to an OD<sub>600nm</sub> of 1.2 at 37° C., and cooled for 1 hour with shaking at 18° C. Afterwards, the cells were induced for 16 h at 18° C. with 0.2 mM IPTG. Cells were harvested and resuspended in 50 mM Tris (pH 8.0), 500 mM NaCl, 5% glycerol, 25 mM imidazole for purification over Ni-NTA resin.

## EXAMPLE 38

## Expression and Purification of c-Src Variants

Hexahistidine-tagged recombinant chicken c-Src (residues 251-533) was prepared in a similar manner to that described in Seeliger MA, et al. *Protein Sci.* 14 (12):3135-3139 with the modifications used by Blair J A, et al. (2007) Structure-guided development of affinity probes for tyrosine kinases using chemical genetics. *Nat Chem Biol* 3(4):229-238. The hexahistidine tag was removed with AcTev protease (Invitrogen) and concentrations were determined spectrophotometrically at 280 nm using an extinction coefficient of 52,370 M<sup>-1</sup>cm<sup>-1</sup>. All mutations were introduced using the site-directed mutagenesis protocol of Zheng L, Baumann U, Raymond J L (2004) An efficient one-step site-directed and site-saturation mutagenesis protocol. *Nucleic Acids Res* 32(14):e115. Protein aliquots were stored at -80° C. in 50 mM Tris (pH 8), 100 mM NaCl, 1 mM DTT and 5% glycerol.

## EXAMPLE 39

## In vitro Kinase Assays

In vitro kinase assays for c-Src variants were performed in 50 mM Tris (pH 8.0), 10 mM MgCl<sub>2</sub> and 1 mg/mL BSA. When obtaining kinetic parameters ( $k_{cat}$ ,  $K_m$ ) kinase and peptide substrate (IYGEFKKK) (SEQ ID NO:51) concentra-

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tions were 2 nM and 500  $\mu$ M, respectively, while ATP concentrations ranged from 2000-0.655  $\mu$ M. Addition of nonradioactive ATP supplemented with  $^{32}$ P ATP (3,000 Ci/mmol, NEN) was used to initiate kinase reactions. Time points were selected such that product formation never exceeded 10%. Reactions were quenched by spotting 3  $\mu$ L quantities onto phosphocellulose sheets (P81, Whatman). Afterwards, the sheets were washed 3 $\times$ 5 minutes in 0.5% phosphoric acid and dried. Radioactivity was measured by phosphorimaging and recorded on a Typhoon fluorescence imager (Molecular Dynamics). Data were plotted as rate ( $\text{min}^{-1}$ ) versus ATP concentration and fitted to the Michaelis-Menten equation,  $v = [(k_{cat}[S])/(K_m + [S])]$ , using Kaleidagraph software (Synergy) to extract kinetic parameters. When obtaining  $\text{IC}_{50}$  values for the inhibitors, 2% (v/v) DMSO was included in kinase reactions. In these cases ATP, peptide, and enzyme concentrations were 15 nM, 100  $\mu$ M and 5 nM, respectively, while inhibitor concentrations ranged from 10,000-0.610 nM. In all cases, a ten-minute preincubation step between the kinase and the inhibitor preceded addition of ATP and a fifteen-minute reaction. The data was fitted to a sigmoidal dose-response curve using Prism 4.0c (GraphPad Software) to obtain  $\text{IC}_{50}$  values.

## EXAMPLE 40

## Crystallization and Data Collection for c-Src-ES1-9

The c-Src-ES1 variant was prepared and purified as described above, run over a 0.22  $\mu$ m PVDF centrifugal filter and diluted to 1.5-3 mg/ml in 50 mM Tris (pH 8.0), 100 mM NaCl, 5% (v/v) glycerol, 1 mM DTT. Compound 9 was freshly dissolved in DMSO and added to the protein solutions (1.5-3 equivalents). After 1.5 hours of incubation at room temperature, the reaction mixtures were spun at 10,000 rpm and the supernatants were collected. Hanging drop crystallization conditions were set up by mixing 1:1 protein and precipitation solutions (100 mM MES (pH 6.5), 50 mM NaOAc, 4-8% PEG 4000). After 24-48 hours at room temperature, thin plate-like crystals were observed. Crystals were cryoprotected in the crystallization solution supplemented with 25% glycerol and stored in liquid nitrogen prior to obtaining diffraction data at beamline 8.2.2 (wavelength of 1.0088 nm, nitrogen gas stream at 100 K) at the Berkeley Lab Advanced Light Source. Data was processed with HKL2000 (HKL Research, Inc.) and Phenix software.

## EXAMPLE 41

## Immunoprecipitation and Assay of MOK

A plasmid encoding full-length mouse MOK with a FLAG-tag for expression in mammalian cells was used. Immunoprecipitation from Cos7 cells was performed using a procedure similar to Miyata Y, Akashi M, Nishida E (1999). Molecular cloning and characterization of a novel member of the MAP kinase superfamily. *Genes Cells* 4(5):299-309, with the following modification: MOK was directly immunoprecipitated on ANTI-FLAG M2 magnetic beads (Sigma-Aldrich). Kinase assays were performed directly on-bead for 60 minutes in 30  $\mu$ L quantities of 50 mM Tris-HCl, 16 mM MOPS, 150 mM NaCl, 10 mM  $\text{MgCl}_2$ , 20 mM  $\beta$ -glycero-

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phosphate, 2 mM EGTA, 0.8 mM sodium orthovanadate, 0.4 mM dithiothreitol, 0.1 mM ATP (supplemented with  $^{32}$ P ATP (3,000 Ci/mmol, NEN)), and 20  $\mu$ g of a protein substrate (myelin basic protein) at a pH of 8. Inhibitors were used at a concentration of 1  $\mu$ M (final DMSO concentration, 2%). Myelin basic protein phosphorylation was analyzed by SDS-PAGE and autoradiography. MOK levels were evaluated by Western blot using HRP conjugated ANTI-FLAG M2 antibody at a 1000:1 dilution.

## EXAMPLE 42

## Inhibition Assays with v-Src Transformed NIH-3T3 Cells

NIH-3T3 cell lines transformed with v-Src gatekeeper variants were prepared using a procedure similar to that in Bishop A C, et al. (1998) Design of allele-specific inhibitors to probe protein kinase signaling. *Curr Biol* 8(5):257-266. Cells were grown to 60-90% confluence in DMEM supplemented with fetal bovine serum (10%), penicillin 'G' (100 units/ml) and streptomycin sulfate (100  $\mu$ g/ml) (PenStrep, UCSF Cell Culture Facility) prior to treatment with kinase inhibitors dissolved in DMSO (final DMSO concentration, 0.5%). Following 1 hour of incubation with inhibitors at 37 $^{\circ}$  C., cells were harvested in lysis buffer (50 mM Tris (pH 7.4), 300 mM NaCl, 5 mM EDTA, 1% triton, 0.02%  $\text{NaN}_3$ , 1 $\times$  complete mini protease inhibitor (Roche), 1 mM PMSF, 1 $\times$ PHOS-stop (Roche), 0.02  $\mu$ M microcystin, 2 mM sodium orthovanadate), normalized for concentration and analyzed by Western blot for global phosphotyrosine levels (4G10, Millipore, 1:1000). Levels of  $\beta$ -actin ( $\beta$ -actin Antibody, Cell Signaling, 1:1000) and v-Src (Src 32G6 rabbit mAb, Cell Signaling, 1:1000) were ascertained by Western blot.

## EXAMPLE 43

## Blockade of v-Src-ES1 Activity in Cells with Electrophilic Inhibitors

I338C (v-Src-ES1, SEQ ID NO:48), I338T (SEQ ID NO:49), I338G (v-Src-AS1, SEQ ID NO:50) and WT v-Src-transformed NIH-3T3 cell lines were generated. Unlike c-Src, v-Src is constitutively active and harbors an isoleucine gatekeeper. The I338T v-Src variant was generated for consistency with the in vitro c-Src studies. For each cell line, global levels of phosphotyrosine were analyzed (FIG. 7). Importantly, the v-Src-ES1 variant was an excellent mimic of WT v-Src, while the activity of v-Src-AS1 (the mutant used in previous chemical genetic studies) was markedly diminished as judged by whole cell phosphotyrosine levels. To determine whether the electrophilic inhibitors function in cells, the v-Src-ES1 and I338T v-Src-transformed cell lines were treated with 9 and 13. Both 9 and 13 inhibited v-Src-ES1 in a dose-dependent manner, while isosteric control compounds (11 and 14) showed no activity (FIG. 4). Furthermore, neither 9 nor 13 inhibited I338T v-Src even at levels as high as 10  $\mu$ M. Collectively, these results suggest that a kinase with a cysteine gatekeeper can be selectively targeted in cells.

## EXAMPLE 44

## Second-site Mutations to Modulate Inhibitor Potency

The design strategy, in order to determine whether further kinase engineering could enhance potency, was to either

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enhance the reactivity of the cysteine by installing nearby hydrophilic/basic residues or to slightly enlarge the area around the cysteine to allow for additional rotational freedom to facilitate optimized thiol-electrophile attack geometry. Accordingly, mutations at Val323—a residue within 4 Å of the gatekeeper (FIG. 2C) in c-Src—were introduced in combination with T338C. Of the double mutants, V323A/T338C (c-Src-ES2) and V323S/T338C (c-Src-ES3) had substantial activity, while V323D/T338C (c-Src-ES4), V323E/T338C (c-Src-ES5), and V323H/T338C (c-Src-ES6) were inactive (Table 4). Enhanced inhibitor potency was observed for both c-Src-ES2 and c-Src-ES3 when treated with 13 (Table 3). In the latter case, a 12-fold improvement was noted relative to c-Src-ES1. Interestingly, the potencies of 3 and 9 were not modulated appreciably upon introduction of the additional mutations. Taken together, these results indicate that the judicious placement of a secondary mutation can be an effective means for modulating inhibitor potency for an ES allele, but that this strategy needs to be evaluated on a case-by-case basis.

## EXAMPLE 45

## Evaluating the Use of a Cysteine Gatekeeper Kinase

A recombinant wild type (WT) and T338C c-Src was generated. The recombinant wild type (WT) and the T338C c-Src were assayed for kinase activity, see Table 8. The  $k_{cat}$  value for T338C c-Src ( $183 \text{ min}^{-1}$ ) closely approximated that of WT ( $159 \text{ min}^{-1}$ ) and was ~3.5-fold greater than that of c-Src-AS1 ( $51.9 \text{ min}^{-1}$ ). The T338C c-Src variant also recapitulated WT in affinity for ATP as determined by the Michaelis constant ( $K_m$ ) values ( $21.9 \text{ }\mu\text{M}$  vs.  $31.9 \text{ }\mu\text{M}$ ), while c-Src-AS1 ( $87.5 \text{ }\mu\text{M}$ ) exhibited ~4-fold loss relative to T338C c-Src. These effects translate to a 14-fold improvement in catalytic efficiency ( $k_{cat}/K_m$ ) for T338C c-Src in relation to c-Src-AS1.

TABLE 8

Kinetic parameters for c-Src variants. Values were determined by fitting data to the Michaelis-Menten equation. Standard errors associated with the fits are reported.			
c-Src Variant	$k_{cat}$ ( $\text{min}^{-1}$ )	$K_m, \text{ATP}$ ( $\mu\text{M}$ )	$k_{cat}/K_m$ ( $\text{min}^{-1} \mu\text{M}^{-1}$ )
WT	$159 \pm 4$	$31.9 \pm 3.0$	$4.99 \pm 0.40$
T338C	$183 \pm 3$	$21.9 \pm 1.7$	$8.34 \pm 0.57$
AS1	$51.9 \pm 1.9$	$87.5 \pm 12.6$	$0.592 \pm 0.072$

Kinetic measurements reveal that in the case of c-Src, the ES1 variant is a mimic of wild type activity. Furthermore, the ES1 variant of v-Src is also a mimic of the wild type, which contains a particularly hydrophobic (isoleucine) gatekeeper. See Tables 9a-9c following. Results on the selectivity of compound 19 in the Invitrogen SelectScreen® Kinase Assay are provided in Tables 9a-9c following. Table 9a is the LanthScreen™ heat map., Table 9b is the Adapta® heat map, and Table 9c is the Z'-lyte™ heat map. Legend for Tables 9a-9c: <40% inhibition (gray); 40%-80% inhibition (white); 80% inhibition (diagonal stripes). Selected results in the

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assays are further provided in FIG. 11. In the table below, Cmpd 3-vs-Q refers to compound 19.

TABLE 9a

Kinase tested		Cmpd 3-vs-Q 1000 nM
ACVR1 (ALK2)	Binding	4
ACVR2B	Binding	19
BMPR1A (ALK3)	Binding	10
CAMKK1 (CAMKKA)	Binding	5
CAMKK2 (CaMKK beta)	Binding	6
CDK8/cyclin C	Binding	4
CDK9/cyclin K	Binding	0
CLK4	Binding	24
DDR1	Binding	0
DDR2	Binding	5
DMPK	Binding	10
EPHA3	Binding	1
EPHA7	Binding	2
KIT V654A	Binding	6
LIMK1	Binding	1
LIMK2	Binding	6
MAP2K1 (MEK1) S218D S222D	Binding	20
MAP2K3 (MEK3)	Binding	6
MAP2K6 (MKK6) S207E T211E	Binding	3
MAP3K10 (MLK2)	Binding	6
MAP3K11 (MLK3)	Binding	1
MAP3K14 (NIK)	Binding	12
MAP3K2 (MEKK2)	Binding	2
MAP3K3 (MEKK3)	Binding	9
MAP3K5 (ASK1)	Binding	3
MAP3K7/MAP3K7IP1 (TAK1-TAB1)	Binding	2
MKNK2 (MNK2)	Binding	36
MLCK (MLCK2)	Binding	36
MYLK (MLCK)	Binding	3
NLK	Binding	5
RIPK2	Binding	52
SLK	Binding	7
STK16 (PKL12)	Binding	5
STK17A (DRAK1)	Binding	21
STK33	Binding	7
TAOK3 (JIK)	Binding	3
TEC	Binding	3
TGFBF1 (ALK5)	Binding	10
TNK2 (ACK)	Binding	10
TTK	Binding	16
WEE1	Binding	14
WNK2	Binding	10
ZAK	Binding	6

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TABLE 9b

Kinase			Cmpd 3-vs-Q 1000 nM
CAMK1 (CaMK1)	Activity	100	-21
CDK7/cyclin H/MNAT1	Activity	Km app	0
CDK9/cyclin T1	Activity	Km app	-24
CHUK (IKK alpha)	Activity	Km app	3
DAPK1	Activity	Km app	6
GSG2 (Haspin)	Activity	Km app	-8
IRAK1	Activity	Km app	6
LRRK2	Activity	Km app	75
LRRK2 G2019S	Activity	Km app	18
NUAK1 (ARK5)	Activity	Km app	24
PI4KA (PI4K alpha)	Activity	10	7
PI4KB (PI4K beta)	Activity	Km app	14
PIK3C2A (PI3K-C2 alpha)			-1
PIK3C2B (PI3K-C2 beta)	Activity	100	65
PIK3C3 (hVPS34)	Activity	Km app	1
PIK3CA/PIK3R1 (p110 alpha/p85 alpha)	Activity	Km app	13
PIK3CD/PIK3R1 (p110 delta/p85 alpha)	Activity	Km app	10
PIK3CG (p110 gamma)	Activity	Km app	17
SPHK1	Activity	Km app	3
SPHK2	Activity	100	-11

TABLE 9c

Kinase			Cmpd 3-vs-Q 1000 nM
ABL1	Activity	Km app	4
ABL1 E255K	Activity	Km app	7
ABL1 G250E	Activity	Km app	2
ABL1 T315I	Activity	Km app	11
ABL1 Y253F	Activity	Km app	12
ABL2 (Arg)	Activity	Km app	10
ACVR1B (ALK4)	Activity	Km app	-1
ADRBK1 (GRK2)	Activity	Km app	13
ADRBK2 (GRK3)	Activity	Km app	-1
AKT1 (PKB alpha)	Activity	Km app	0
AKT2 (PKB beta)	Activity	Km app	4
AKT3 (PKB gamma)	Activity	Km app	4
ALK	Activity	Km app	3
AMPK A1/B1/G1	Activity	Km app	18
AMPK A2/B1/G1	Activity	Km app	15
AURKA (Aurora A)	Activity	Km app	12

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TABLE 9c-continued

5	AURKB (Aurora B)	Activity	Km app	26
	AURKC (Aurora C)	Activity	Km app	1
	AXL	Activity	Km app	19
	BLK	Activity	Km app	24
	BMX	Activity	Km app	24
10	BRAF	Activity	100	4
	BRAF V599E	Activity	100	13
	BRSK1 (SAD1)	Activity	Km app	35
	BTK	Activity	Km app	59
15	CAMK1D (CaMKI delta)	Activity	Km app	15
	CAMK2A (CaMKII alpha)	Activity	Km app	4
	CAMK2B (CaMKII beta)	Activity	Km app	5
	CAMK2D (CaMKII delta)	Activity	Km app	10
20	CAMK4 (CaMKIV)	Activity	Km app	10
	CDC42 BPA (MRCKA)	Activity	Km app	23
	CDC42 BPB (MRCKB)	Activity	Km app	-4
	CDK1/cyclin B	Activity	Km app	3
25	CDK2/cyclin A	Activity	Km app	4
	CDK5/p25	Activity	Km app	10
30				
	CDK5/p35	Activity	Km app	5
	CHEK1 (CHK1)	Activity	Km app	-6
35	CHEK2 (CHK2)	Activity	Km app	53
	CLK1	Activity	Km app	11
	CLK2	Activity	Km app	-3
40				
45	CLK3	Activity	Km app	6
	CSF1R (FMS)	Activity	Km app	3
	CSK	Activity	Km app	12
	CSNK1A1 (CK1 alpha 1)	Activity	Km app	0
50	CSNK1D (CK1 delta)	Activity	Km app	5
	CSNK1E (CK1 epsilon)	Activity	Km app	6
	CSNK1G1 (CK1 gamma 1)	Activity	Km app	12
55	CSNK1G2 (CK1 gamma 2)	Activity	Km app	31
	CSNK1G3 (CK1 gamma 3)	Activity	Km app	31
60	CSNK2A1 (CK2 alpha 1)	Activity	Km app	13
	CSNK2A2 (CK2 alpha 2)	Activity	Km app	-1
	DAPK3 (ZIPK)	Activity	Km app	0
	DCAMKL2 (DCK2)	Activity	Km app	5
	DNA-PK	Activity	Km app	25
65	DYRK1A	Activity	Km app	-2

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TABLE 9c-continued

DYRK1B	Activity	Km app	3
DYRK3	Activity	Km app	5
DYRK4	Activity	Km app	3
EEF2K	Activity	Km app	5
EGFR (ErbB1)	Activity	Km app	
EGFR (ErbB1) L858R	Activity	Km app	69
EGFR (ErbB1) L861Q	Activity	Km app	76
EGFR (ErbB1) T790M	Activity	Km app	27
EGFR (ErbB1) T790M L858R	Activity	Km app	41
EPHA1	Activity	Km app	19
EPHA2	Activity	Km app	4
EPHA4	Activity	Km app	8
EPHA5	Activity	Km app	7
EPHA8	Activity	Km app	11
EPHB1	Activity	Km app	8
EPHB2	Activity	Km app	15
EPHB3	Activity	Km app	8
EPHB4	Activity	Km app	7
ERBB2 (HER2)	Activity	Km app	66
ERBB4 (HER4)	Activity	Km app	80
FER	Activity	Km app	6
FES (FPS)	Activity	Km app	16
FGFR1	Activity	Km app	8
FGFR2	Activity	Km app	14
FGFR3	Activity	Km app	11
FGFR3 K650E	Activity	Km app	18
FGFR4	Activity	Km app	14
FGR	Activity	Km app	33
FLT1 (VEGFR1)	Activity	Km app	2
FLT3	Activity	Km app	32
FLT3 D835Y	Activity	Km app	
FLT4 (VEGFR3)	Activity	Km app	34
FRAP1 (mTOR)	Activity	Km app	7
FRK (PTK5)	Activity	Km app	11
FYN	Activity	Km app	3
GRK4	Activity	Km app	15
GRK5	Activity	Km app	49
GRK6	Activity	Km app	27
GRK7	Activity	Km app	2
GSK3A (GSK3 alpha)	Activity	Km app	2
GSK3B (GSK3 beta)	Activity	Km app	4
HCK	Activity	Km app	13
HIPK1 (Myak)	Activity	Km app	3
HIPK2	Activity	Km app	4
HIPK3 (YAK1)	Activity	Km app	3
HIPK4	Activity	Km app	17
IGF1R	Activity	Km app	5
IKBKB (IKK beta)	Activity	Km app	13
IKBKE (IKK epsilon)	Activity	Km app	10
INSR	Activity	Km app	0

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TABLE 9c-continued

INSRR (IRR)	Activity	Km app	11
IRAK4	Activity	Km app	3
ITK	Activity	Km app	3
JAK1	Activity	Km app	14
JAK2	Activity	Km app	0
JAK2 JH1 JH2	Activity	Km app	5
JAK2 JH1 JH2 V617F	Activity	Km app	4
JAK3	Activity	Km app	37
KDR (VEGFR2)	Activity	Km app	6
KIT	Activity	Km app	7
KIT T670I	Activity	Km app	5
LCK	Activity	Km app	0
LTK (TYK1)	Activity	Km app	4
LYN A	Activity	Km app	21
LYN B	Activity	Km app	25
MAP2K1 (MEK1)	Activity	100	3
MAP2K2 (MEK2)	Activity	100	13
MAP2K6 (MKK6)	Activity	100	17
MAP3K8 (COT)	Activity	100	4
MAP3K9 (MLK1)	Activity	Km app	3
MAP4K2 (GCK)	Activity	Km app	13
MAP4K4 (HGK)	Activity	Km app	23
MAP4K5 (KHS1)	Activity	Km app	23
MAPK1 (ERK2)	Activity	Km app	2
MAPK10 (JNK3)	Activity	100	2
MAPK11 (p38 beta)	Activity	Km app	14
MAPK12 (p38 gamma)	Activity	Km app	10
MAPK13 (p38 delta)	Activity	Km app	5
MAPK14 (p38 alpha)	Activity	100	24
MAPK14 (p38 alpha) Direct	Activity	Km app	11
MAPK3 (ERK1)	Activity	Km app	10
MAPK8 (JNK1)	Activity	100	19
MAPK9 (JNK2)	Activity	100	8
MAPKAPK2	Activity	Km app	6
MAPKAPK3	Activity	Km app	5
MAPKAPK5 (PRAK)	Activity	Km app	7
MARK1 (MARK)	Activity	Km app	1
MARK2	Activity	Km app	2
MARK3	Activity	Km app	5
MARK4	Activity	Km app	1

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TABLE 9c-continued

MATK (HYL)	Activity	Km app	5
MELK	Activity	Km app	20
MERTK (cMER)	Activity	Km app	12
MET (cMet)	Activity	Km app	3
MET M1250T	Activity	Km app	8
MINK1	Activity	Km app	17
MKNK1 (MKNK1)	Activity	Km app	40
MST1R (RON)	Activity	Km app	11
MST4	Activity	Km app	15
MUSK	Activity	Km app	14
MYLK2 (skMLCK)	Activity	Km app	13
NEK1	Activity	Km app	-2
NEK2	Activity	Km app	-1
NEK4	Activity	Km app	22
NEK6	Activity	Km app	6

NEK7	Activity	Km app	6
NEK9	Activity	Km app	10
NTRK1 (TRKA)	Activity	Km app	18
NTRK2 (TRKB)	Activity	Km app	8
NTRK3 (TRKC)	Activity	Km app	3
PAK1	Activity	Km app	14
PAK2 (PAK65)	Activity	Km app	12
PAK3	Activity	Km app	3
PAK4	Activity	Km app	-4
PAK6	Activity	Km app	8
PAK7 (KIAA1264)	Activity	Km app	9
PASK	Activity	Km app	8
PDGFRA (PDGFR alpha)	Activity	Km app	18

PDGFRA D842V	Activity	Km app	22
PDGFRA T674I	Activity	Km app	25
PDGFRA V561D	Activity	Km app	45
PDGFRB (PDGFR beta)	Activity	Km app	8
PDK1	Activity	100	16
PDK1 Direct	Activity	Km app	0
PHKG1	Activity	Km app	10
PHKG2	Activity	Km app	4
PIM1	Activity	Km app	12
PIM2	Activity	Km app	0
PKN1 (PRK1)	Activity	Km app	19
PLK1	Activity	Km app	-1
PLK2	Activity	Km app	10

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TABLE 9c-continued

PLK3	Activity	Km app	-2
PRKACA (PKA)	Activity	Km app	0
PRKCA (PKC alpha)	Activity	Km app	14
PRKCB1 (PKC beta I)	Activity	Km app	10
PRKCB2 (PKC beta II)	Activity	Km app	5
PRKCD (PKC delta)	Activity	Km app	15
PRKCE (PKC epsilon)	Activity	Km app	17
PRKCG (PKC gamma)	Activity	Km app	14
PRKCH (PKC eta)	Activity	Km app	15
PRKCI (PKC iota)	Activity	Km app	10
PRKCN (PKD3)	Activity	Km app	10
PRKCQ (PKC theta)	Activity	Km app	13
PRKCZ (PKC zeta)	Activity	Km app	6
PRKD1 (PKC mu)	Activity	Km app	12

PRKD2 (PKD2)	Activity	Km app	12
PRKG1	Activity	Km app	2
PRKG2 (PKG2)	Activity	Km app	-1
PRKX	Activity	Km app	3
PTK2 (FAK)	Activity	Km app	9
PTK2B (FAK2)	Activity	Km app	4
PTK6 (Brk)	Activity	Km app	9
RAF1 (cRAF) Y340D	Activity	100	21
Y341D			
RET	Activity	Km app	19
RET V804L	Activity	Km app	12
RET Y791F	Activity	Km app	25
ROCK1	Activity	Km app	-3

ROCK2	Activity	Km app	13
ROS1	Activity	Km app	40
RPS6KA1 (RSK1)	Activity	Km app	6
RPS6KA2 (RSK3)	Activity	Km app	26
RPS6KA3 (RSK2)	Activity	Km app	9
RPS6KA4 (MSK2)	Activity	Km app	6
RPS6KA5 (MSK1)	Activity	Km app	0
RPS6KA6 (RSK4)	Activity	Km app	49
RPS6KB1 (p70S6K)	Activity	Km app	10
SGK (SGK1)	Activity	Km app	6
SGK2	Activity	Km app	8
SGKL (SGK3)	Activity	Km app	4
SNF1LK2	Activity	Km app	4
SRC	Activity	Km app	4

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TABLE 9c-continued

SRC N1	Activity	Km app	15
SRMS (Srm)	Activity	Km app	71
SRPK1	Activity	Km app	3
SRPK2	Activity	Km app	10
STK22B (TSSK2)	Activity	Km app	2
STK22D (TSSK1)	Activity	Km app	13
STK23 (MSSK1)	Activity	Km app	14
STK24 (MST3)	Activity	Km app	11
STK25 (YSK1)	Activity	Km app	5
STK3 (MST2)	Activity	Km app	-9
STK4 (MST1)	Activity	Km app	3
SYK	Activity	Km app	-2
TAOK2 (TAO1)	Activity	Km app	2
TBK1	Activity	Km app	-2

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TABLE 9c-continued

TEK (Tie2)	Activity	Km app	-7
TXK	Activity	Km app	78
TYK2	Activity	Km app	-4
TYRO3 (RSE)	Activity	Km app	22
YES1	Activity	Km app	30
ZAP70	Activity	Km app	10

5

10

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Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, one of skill in the art will appreciate that certain changes and modifications may be practiced within the scope of the appended claims. In addition, each reference provided herein is incorporated by reference in its entirety to the same extent as if each reference was individually incorporated by reference. Where a conflict exists between the instant application and a reference provided herein, the instant application shall dominate.

## SEQUENCE LISTING

&lt;160&gt; NUMBER OF SEQ ID NOS: 80

&lt;210&gt; SEQ ID NO 1

&lt;211&gt; LENGTH: 918

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial sequence

&lt;220&gt; FEATURE:

<223> OTHER INFORMATION: synthetic DNA construct for T338C c-src  
(251-533)

&lt;400&gt; SEQUENCE: 1

```

catcatcatc atcatcacga ttacgatatc ccaacgaccg aaaaccttta cttccagggc   60
catatgcaga cccagggact cgccaaggac gcgtgggaaa tcccccgga gtcgtgcgg   120
ctggagggtga agctggggca gggctgcttt ggagaggtct g gatggggac ctggaacggc   180
accaccagag tggccataaa gactctgaag cccggcacca tgtccccga ggccttcctg   240
caggaagccc aagtgatgaa gaagctccgg catgagaagc tggttcagct gtacgcagtg   300
gtgtcggaag agcccatcta catcgtctgt gagtacatga gcaaggggag cctcctggat   360
ttcctgaagg gagagatggg caagtacctg cggtgccac agctcgtcga tatggctgct   420
cagattgcat ccggcatggc ctatgtggag aggatgaact acgtgcaccg agacctgcgg   480
gcggccaaca tcctggtggg ggagaacctg gtgtgcaagg tggctgactt tgggctggca   540
cgctcatcg aggacaacga gtacacagca cggaagggtg ccaagttccc catcaagtgg   600
acagcccccg aggcagccct ctatggccgg ttcaccatca agtcggatgt ctggtccttc   660
ggcatcctgc tgactgagct gaccaccaag ggccgggtgc cataccagg gatgtcaac   720
agggagggtgc tggaccaggt ggagaggggc taccgcatgc cctgccgcc ccagtgcccc   780
gagtcgctgc atgacctcat gtgccagtgc tggcggaagg accctgagga gcggcccaat   840
tttgagtacc tgcaggcctt cctggaggac tacttcacct cgacagagcc ccagtaccag   900

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-continued

cctggagaga acctatag

918

<210> SEQ ID NO 2  
 <211> LENGTH: 305  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: synthetic T338C c-src (251-533) protein

&lt;400&gt; SEQUENCE: 2

His His His His His His Asp Tyr Asp Ile Pro Thr Thr Glu Asn Leu  
 1 5 10 15  
 Tyr Phe Gln Gly His Met Gln Thr Gln Gly Leu Ala Lys Asp Ala Trp  
 20 25 30  
 Glu Ile Pro Arg Glu Ser Leu Arg Leu Glu Val Lys Leu Gly Gln Gly  
 35 40 45  
 Cys Phe Gly Glu Val Trp Met Gly Thr Trp Asn Gly Thr Thr Arg Val  
 50 55 60  
 Ala Ile Lys Thr Leu Lys Pro Gly Thr Met Ser Pro Glu Ala Phe Leu  
 65 70 75 80  
 Gln Glu Ala Gln Val Met Lys Lys Leu Arg His Glu Lys Leu Val Gln  
 85 90 95  
 Leu Tyr Ala Val Val Ser Glu Glu Pro Ile Tyr Ile Val Cys Glu Tyr  
 100 105 110  
 Met Ser Lys Gly Ser Leu Leu Asp Phe Leu Lys Gly Glu Met Gly Lys  
 115 120 125  
 Tyr Leu Arg Leu Pro Gln Leu Val Asp Met Ala Ala Gln Ile Ala Ser  
 130 135 140  
 Gly Met Ala Tyr Val Glu Arg Met Asn Tyr Val His Arg Asp Leu Arg  
 145 150 155 160  
 Ala Ala Asn Ile Leu Val Gly Glu Asn Leu Val Cys Lys Val Ala Asp  
 165 170 175  
 Phe Gly Leu Ala Arg Leu Ile Glu Asp Asn Glu Tyr Thr Ala Arg Gln  
 180 185 190  
 Gly Ala Lys Phe Pro Ile Lys Trp Thr Ala Pro Glu Ala Ala Leu Tyr  
 195 200 205  
 Gly Arg Phe Thr Ile Lys Ser Asp Val Trp Ser Phe Gly Ile Leu Leu  
 210 215 220  
 Thr Glu Leu Thr Thr Lys Gly Arg Val Pro Tyr Pro Gly Met Val Asn  
 225 230 235 240  
 Arg Glu Val Leu Asp Gln Val Glu Arg Gly Tyr Arg Met Pro Cys Pro  
 245 250 255  
 Pro Glu Cys Pro Glu Ser Leu His Asp Leu Met Cys Gln Cys Trp Arg  
 260 265 270  
 Lys Asp Pro Glu Glu Arg Pro Thr Phe Glu Tyr Leu Gln Ala Phe Leu  
 275 280 285  
 Glu Asp Tyr Phe Thr Ser Thr Glu Pro Gln Tyr Gln Pro Gly Glu Asn  
 290 295 300  
 Leu  
 305

<210> SEQ ID NO 3  
 <211> LENGTH: 533  
 <212> TYPE: PRT  
 <213> ORGANISM: Gallus gallus  
 <220> FEATURE:  
 <223> OTHER INFORMATION: proto-oncogene c-Src

-continued

&lt;400&gt; SEQUENCE: 3

Met Gly Ser Ser Lys Ser Lys Pro Lys Asp Pro Ser Gln Arg Arg Arg  
 1 5 10 15  
 Ser Leu Glu Pro Pro Asp Ser Thr His His Gly Gly Phe Pro Ala Ser  
 20 25 30  
 Gln Thr Pro Asn Lys Thr Ala Ala Pro Asp Thr His Arg Thr Pro Ser  
 35 40 45  
 Arg Ser Phe Gly Thr Val Ala Thr Glu Pro Lys Leu Phe Gly Gly Phe  
 50 55 60  
 Asn Thr Ser Asp Thr Val Thr Ser Pro Gln Arg Ala Gly Ala Leu Ala  
 65 70 75 80  
 Gly Gly Val Thr Thr Phe Val Ala Leu Tyr Asp Tyr Glu Ser Arg Thr  
 85 90 95  
 Glu Thr Asp Leu Ser Phe Lys Lys Gly Glu Arg Leu Gln Ile Val Asn  
 100 105 110  
 Asn Thr Glu Gly Asp Trp Trp Leu Ala His Ser Leu Thr Thr Gly Gln  
 115 120 125  
 Thr Gly Tyr Ile Pro Ser Asn Tyr Val Ala Pro Ser Asp Ser Ile Gln  
 130 135 140  
 Ala Glu Glu Trp Tyr Phe Gly Lys Ile Thr Arg Arg Glu Ser Glu Arg  
 145 150 155 160  
 Leu Leu Leu Asn Pro Glu Asn Pro Arg Gly Thr Phe Leu Val Arg Glu  
 165 170 175  
 Ser Glu Thr Thr Lys Gly Ala Tyr Cys Leu Ser Val Ser Asp Phe Asp  
 180 185 190  
 Asn Ala Lys Gly Leu Asn Val Lys His Tyr Lys Ile Arg Lys Leu Asp  
 195 200 205  
 Ser Gly Gly Phe Tyr Ile Thr Ser Arg Thr Gln Phe Ser Ser Leu Gln  
 210 215 220  
 Gln Leu Val Ala Tyr Tyr Ser Lys His Ala Asp Gly Leu Cys His Arg  
 225 230 235 240  
 Leu Thr Asn Val Cys Pro Thr Ser Lys Pro Gln Thr Gln Gly Leu Ala  
 245 250 255  
 Lys Asp Ala Trp Glu Ile Pro Arg Glu Ser Leu Arg Leu Glu Val Lys  
 260 265 270  
 Leu Gly Gln Gly Cys Phe Gly Glu Val Trp Met Gly Thr Trp Asn Gly  
 275 280 285  
 Thr Thr Arg Val Ala Ile Lys Thr Leu Lys Pro Gly Thr Met Ser Pro  
 290 295 300  
 Glu Ala Phe Leu Gln Glu Ala Gln Val Met Lys Lys Leu Arg His Glu  
 305 310 315 320  
 Lys Leu Val Gln Leu Tyr Ala Val Val Ser Glu Glu Pro Ile Tyr Ile  
 325 330 335  
 Val Thr Glu Tyr Met Ser Lys Gly Ser Leu Leu Asp Phe Leu Lys Gly  
 340 345 350  
 Glu Met Gly Lys Tyr Leu Arg Leu Pro Gln Leu Val Asp Met Ala Ala  
 355 360 365  
 Gln Ile Ala Ser Gly Met Ala Tyr Val Glu Arg Met Asn Tyr Val His  
 370 375 380  
 Arg Asp Leu Arg Ala Ala Asn Ile Leu Val Gly Glu Asn Leu Val Cys  
 385 390 395 400  
 Lys Val Ala Asp Phe Gly Leu Ala Arg Leu Ile Glu Asp Asn Glu Tyr

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Thr	Ala	Arg	Gln	Gly	Ala	Lys	Phe	Pro	Ile	Lys	Trp	Thr	Ala	Pro	Glu
			420					425					430		
Ala	Ala	Leu	Tyr	Gly	Arg	Phe	Thr	Ile	Lys	Ser	Asp	Val	Trp	Ser	Phe
		435				440					445				
Gly	Ile	Leu	Leu	Thr	Glu	Leu	Thr	Thr	Lys	Gly	Arg	Val	Pro	Tyr	Pro
	450					455					460				
Gly	Met	Val	Asn	Arg	Glu	Val	Leu	Asp	Gln	Val	Glu	Arg	Gly	Tyr	Arg
465					470					475					480
Met	Pro	Cys	Pro	Pro	Glu	Cys	Pro	Glu	Ser	Leu	His	Asp	Leu	Met	Cys
			485						490					495	
Gln	Cys	Trp	Arg	Lys	Asp	Pro	Glu	Glu	Arg	Pro	Thr	Phe	Glu	Tyr	Leu
			500					505					510		
Gln	Ala	Phe	Leu	Glu	Asp	Tyr	Phe	Thr	Ser	Thr	Glu	Pro	Gln	Tyr	Gln
		515					520					525			
Pro	Gly	Glu	Asn	Leu											
	530														

&lt;210&gt; SEQ ID NO 4

&lt;211&gt; LENGTH: 283

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial sequence

&lt;220&gt; FEATURE:

<223> OTHER INFORMATION: synthetic Gallus gallus proto-oncogene c-Src  
(251-533)

&lt;400&gt; SEQUENCE: 4

Gln	Thr	Gln	Gly	Leu	Ala	Lys	Asp	Ala	Trp	Glu	Ile	Pro	Arg	Glu	Ser
1				5					10					15	
Leu	Arg	Leu	Glu	Val	Lys	Leu	Gly	Gln	Gly	Cys	Phe	Gly	Glu	Val	Trp
		20						25					30		
Met	Gly	Thr	Trp	Asn	Gly	Thr	Thr	Arg	Val	Ala	Ile	Lys	Thr	Leu	Lys
		35				40						45			
Pro	Gly	Thr	Met	Ser	Pro	Glu	Ala	Phe	Leu	Gln	Glu	Ala	Gln	Val	Met
	50					55					60				
Lys	Lys	Leu	Arg	His	Glu	Lys	Leu	Val	Gln	Leu	Tyr	Ala	Val	Val	Ser
65					70					75					80
Glu	Glu	Pro	Ile	Tyr	Ile	Val	Thr	Glu	Tyr	Met	Ser	Lys	Gly	Ser	Leu
			85						90					95	
Leu	Asp	Phe	Leu	Lys	Gly	Glu	Met	Gly	Lys	Tyr	Leu	Arg	Leu	Pro	Gln
		100						105					110		
Leu	Val	Asp	Met	Ala	Ala	Gln	Ile	Ala	Ser	Gly	Met	Ala	Tyr	Val	Glu
	115					120					125				
Arg	Met	Asn	Tyr	Val	His	Arg	Asp	Leu	Arg	Ala	Ala	Asn	Ile	Leu	Val
	130					135					140				
Gly	Glu	Asn	Leu	Val	Cys	Lys	Val	Ala	Asp	Phe	Gly	Leu	Ala	Arg	Leu
145					150					155					160
Ile	Glu	Asp	Asn	Glu	Tyr	Thr	Ala	Arg	Gln	Gly	Ala	Lys	Phe	Pro	Ile
			165						170					175	
Lys	Trp	Thr	Ala	Pro	Glu	Ala	Ala	Leu	Tyr	Gly	Arg	Phe	Thr	Ile	Lys
		180						185					190		
Ser	Asp	Val	Trp	Ser	Phe	Gly	Ile	Leu	Leu	Thr	Glu	Leu	Thr	Thr	Lys
	195						200					205			
Gly	Arg	Val	Pro	Tyr	Pro	Gly	Met	Val	Asn	Arg	Glu	Val	Leu	Asp	Gln
	210					215						220			

-continued

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Val	Glu	Arg	Gly	Tyr	Arg	Met	Pro	Cys	Pro	Pro	Glu	Cys	Pro	Glu	Ser
225					230					235					240
Leu	His	Asp	Leu	Met	Cys	Gln	Cys	Trp	Arg	Lys	Asp	Pro	Glu	Glu	Arg
			245					250						255	
Pro	Thr	Phe	Glu	Tyr	Leu	Gln	Ala	Phe	Leu	Glu	Asp	Tyr	Phe	Thr	Ser
		260					265						270		
Thr	Glu	Pro	Gln	Tyr	Gln	Pro	Gly	Glu	Asn	Leu					
	275						280								

<210> SEQ ID NO 5  
 <211> LENGTH: 286  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: synthetic Gallus gallus proto-oncogene c-Src  
 (251-533) with GHM at N-terminal

<400> SEQUENCE: 5

Gly	His	Met	Gln	Thr	Gln	Gly	Leu	Ala	Lys	Asp	Ala	Trp	Glu	Ile	Pro
1			5						10					15	
Arg	Glu	Ser	Leu	Arg	Leu	Glu	Val	Lys	Leu	Gly	Gln	Gly	Cys	Phe	Gly
		20						25					30		
Glu	Val	Trp	Met	Gly	Thr	Trp	Asn	Gly	Thr	Thr	Arg	Val	Ala	Ile	Lys
		35					40					45			
Thr	Leu	Lys	Pro	Gly	Thr	Met	Ser	Pro	Glu	Ala	Phe	Leu	Gln	Glu	Ala
	50					55					60				
Gln	Val	Met	Lys	Lys	Leu	Arg	His	Glu	Lys	Leu	Val	Gln	Leu	Tyr	Ala
65					70					75					80
Val	Val	Ser	Glu	Glu	Pro	Ile	Tyr	Ile	Val	Thr	Glu	Tyr	Met	Ser	Lys
			85					90						95	
Gly	Ser	Leu	Leu	Asp	Phe	Leu	Lys	Gly	Glu	Met	Gly	Lys	Tyr	Leu	Arg
		100						105					110		
Leu	Pro	Gln	Leu	Val	Asp	Met	Ala	Ala	Gln	Ile	Ala	Ser	Gly	Met	Ala
		115					120					125			
Tyr	Val	Glu	Arg	Met	Asn	Tyr	Val	His	Arg	Asp	Leu	Arg	Ala	Ala	Asn
	130					135					140				
Ile	Leu	Val	Gly	Glu	Asn	Leu	Val	Cys	Lys	Val	Ala	Asp	Phe	Gly	Leu
145					150					155					160
Ala	Arg	Leu	Ile	Glu	Asp	Asn	Glu	Tyr	Thr	Ala	Arg	Gln	Gly	Ala	Lys
			165						170					175	
Phe	Pro	Ile	Lys	Trp	Thr	Ala	Pro	Glu	Ala	Ala	Leu	Tyr	Gly	Arg	Phe
			180					185					190		
Thr	Ile	Lys	Ser	Asp	Val	Trp	Ser	Phe	Gly	Ile	Leu	Leu	Thr	Glu	Leu
	195						200					205			
Thr	Thr	Lys	Gly	Arg	Val	Pro	Tyr	Pro	Gly	Met	Val	Asn	Arg	Glu	Val
	210					215					220				
Leu	Asp	Gln	Val	Glu	Arg	Gly	Tyr	Arg	Met	Pro	Cys	Pro	Pro	Glu	Cys
225					230					235					240
Pro	Glu	Ser	Leu	His	Asp	Leu	Met	Cys	Gln	Cys	Trp	Arg	Lys	Asp	Pro
			245						250					255	
Glu	Glu	Arg	Pro	Thr	Phe	Glu	Tyr	Leu	Gln	Ala	Phe	Leu	Glu	Asp	Tyr
			260						265					270	
Phe	Thr	Ser	Thr	Glu	Pro	Gln	Tyr	Gln	Pro	Gly	Glu	Asn	Leu		
	275						280					285			

<210> SEQ ID NO 6

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<211> LENGTH: 283
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic Gallus gallus proto-oncogene
[T338X]c-Src (251-533)
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: (88)..(88)
<223> OTHER INFORMATION: Xaa = any naturally occurring amino acid

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<400> SEQUENCE: 6

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Gln Thr Gln Gly Leu Ala Lys Asp Ala Trp Glu Ile Pro Arg Glu Ser
 1             5             10            15

Leu Arg Leu Glu Val Lys Leu Gly Gln Gly Cys Phe Gly Glu Val Trp
 20            25            30

Met Gly Thr Trp Asn Gly Thr Thr Arg Val Ala Ile Lys Thr Leu Lys
 35            40            45

Pro Gly Thr Met Ser Pro Glu Ala Phe Leu Gln Glu Ala Gln Val Met
 50            55            60

Lys Lys Leu Arg His Glu Lys Leu Val Gln Leu Tyr Ala Val Val Ser
 65            70            75            80

Glu Glu Pro Ile Tyr Ile Val Xaa Glu Tyr Met Ser Lys Gly Ser Leu
 85            90            95

Leu Asp Phe Leu Lys Gly Glu Met Gly Lys Tyr Leu Arg Leu Pro Gln
100            105            110

Leu Val Asp Met Ala Ala Gln Ile Ala Ser Gly Met Ala Tyr Val Glu
115            120            125

Arg Met Asn Tyr Val His Arg Asp Leu Arg Ala Ala Asn Ile Leu Val
130            135            140

Gly Glu Asn Leu Val Cys Lys Val Ala Asp Phe Gly Leu Ala Arg Leu
145            150            155            160

Ile Glu Asp Asn Glu Tyr Thr Ala Arg Gln Gly Ala Lys Phe Pro Ile
165            170            175

Lys Trp Thr Ala Pro Glu Ala Ala Leu Tyr Gly Arg Phe Thr Ile Lys
180            185            190

Ser Asp Val Trp Ser Phe Gly Ile Leu Leu Thr Glu Leu Thr Thr Lys
195            200            205

Gly Arg Val Pro Tyr Pro Gly Met Val Asn Arg Glu Val Leu Asp Gln
210            215            220

Val Glu Arg Gly Tyr Arg Met Pro Cys Pro Pro Glu Cys Pro Glu Ser
225            230            235            240

Leu His Asp Leu Met Cys Gln Cys Trp Arg Lys Asp Pro Glu Glu Arg
245            250            255

Pro Thr Phe Glu Tyr Leu Gln Ala Phe Leu Glu Asp Tyr Phe Thr Ser
260            265            270

Thr Glu Pro Gln Tyr Gln Pro Gly Glu Asn Leu
275            280

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<210> SEQ ID NO 7
<211> LENGTH: 286
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic Gallus gallus proto-oncogene
GHM-[T338X]c-Src (251-533) (GHM at N-terminal)
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: (91)..(91)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid

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&lt;400&gt; SEQUENCE: 7

Gly His Met Gln Thr Gln Gly Leu Ala Lys Asp Ala Trp Glu Ile Pro  
 1 5 10 15  
 Arg Glu Ser Leu Arg Leu Glu Val Lys Leu Gly Gln Gly Cys Phe Gly  
 20 25 30  
 Glu Val Trp Met Gly Thr Trp Asn Gly Thr Thr Arg Val Ala Ile Lys  
 35 40 45  
 Thr Leu Lys Pro Gly Thr Met Ser Pro Glu Ala Phe Leu Gln Glu Ala  
 50 55 60  
 Gln Val Met Lys Lys Leu Arg His Glu Lys Leu Val Gln Leu Tyr Ala  
 65 70 75 80  
 Val Val Ser Glu Glu Pro Ile Tyr Ile Val Xaa Glu Tyr Met Ser Lys  
 85 90 95  
 Gly Ser Leu Leu Asp Phe Leu Lys Gly Glu Met Gly Lys Tyr Leu Arg  
 100 105 110  
 Leu Pro Gln Leu Val Asp Met Ala Ala Gln Ile Ala Ser Gly Met Ala  
 115 120 125  
 Tyr Val Glu Arg Met Asn Tyr Val His Arg Asp Leu Arg Ala Ala Asn  
 130 135 140  
 Ile Leu Val Gly Glu Asn Leu Val Cys Lys Val Ala Asp Phe Gly Leu  
 145 150 155 160  
 Ala Arg Leu Ile Glu Asp Asn Glu Tyr Thr Ala Arg Gln Gly Ala Lys  
 165 170 175  
 Phe Pro Ile Lys Trp Thr Ala Pro Glu Ala Ala Leu Tyr Gly Arg Phe  
 180 185 190  
 Thr Ile Lys Ser Asp Val Trp Ser Phe Gly Ile Leu Leu Thr Glu Leu  
 195 200 205  
 Thr Thr Lys Gly Arg Val Pro Tyr Pro Gly Met Val Asn Arg Glu Val  
 210 215 220  
 Leu Asp Gln Val Glu Arg Gly Tyr Arg Met Pro Cys Pro Pro Glu Cys  
 225 230 235 240  
 Pro Glu Ser Leu His Asp Leu Met Cys Gln Cys Trp Arg Lys Asp Pro  
 245 250 255  
 Glu Glu Arg Pro Thr Phe Glu Tyr Leu Gln Ala Phe Leu Glu Asp Tyr  
 260 265 270  
 Phe Thr Ser Thr Glu Pro Gln Tyr Gln Pro Gly Glu Asn Leu  
 275 280 285

&lt;210&gt; SEQ ID NO 8

&lt;211&gt; LENGTH: 283

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial sequence

&lt;220&gt; FEATURE:

<223> OTHER INFORMATION: synthetic Gallus gallus proto-oncogene  
 [T338C]c-Src (251-533) (c-Src "ES1")

&lt;400&gt; SEQUENCE: 8

Gln Thr Gln Gly Leu Ala Lys Asp Ala Trp Glu Ile Pro Arg Glu Ser  
 1 5 10 15  
 Leu Arg Leu Glu Val Lys Leu Gly Gln Gly Cys Phe Gly Glu Val Trp  
 20 25 30  
 Met Gly Thr Trp Asn Gly Thr Thr Arg Val Ala Ile Lys Thr Leu Lys  
 35 40 45  
 Pro Gly Thr Met Ser Pro Glu Ala Phe Leu Gln Glu Ala Gln Val Met  
 50 55 60

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Lys Lys Leu Arg His Glu Lys Leu Val Gln Leu Tyr Ala Val Val Ser  
 65 70 75 80  
 Glu Glu Pro Ile Tyr Ile Val Cys Glu Tyr Met Ser Lys Gly Ser Leu  
 85 90 95  
 Leu Asp Phe Leu Lys Gly Glu Met Gly Lys Tyr Leu Arg Leu Pro Gln  
 100 105 110  
 Leu Val Asp Met Ala Ala Gln Ile Ala Ser Gly Met Ala Tyr Val Glu  
 115 120 125  
 Arg Met Asn Tyr Val His Arg Asp Leu Arg Ala Ala Asn Ile Leu Val  
 130 135 140  
 Gly Glu Asn Leu Val Cys Lys Val Ala Asp Phe Gly Leu Ala Arg Leu  
 145 150 155 160  
 Ile Glu Asp Asn Glu Tyr Thr Ala Arg Gln Gly Ala Lys Phe Pro Ile  
 165 170 175  
 Lys Trp Thr Ala Pro Glu Ala Ala Leu Tyr Gly Arg Phe Thr Ile Lys  
 180 185 190  
 Ser Asp Val Trp Ser Phe Gly Ile Leu Leu Thr Glu Leu Thr Thr Lys  
 195 200 205  
 Gly Arg Val Pro Tyr Pro Gly Met Val Asn Arg Glu Val Leu Asp Gln  
 210 215 220  
 Val Glu Arg Gly Tyr Arg Met Pro Cys Pro Pro Glu Cys Pro Glu Ser  
 225 230 235 240  
 Leu His Asp Leu Met Cys Gln Cys Trp Arg Lys Asp Pro Glu Glu Arg  
 245 250 255  
 Pro Thr Phe Glu Tyr Leu Gln Ala Phe Leu Glu Asp Tyr Phe Thr Ser  
 260 265 270  
 Thr Glu Pro Gln Tyr Gln Pro Gly Glu Asn Leu  
 275 280

<210> SEQ ID NO 9  
 <211> LENGTH: 286  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: synthetic Gallus gallus proto-oncogene  
 GHM-[T338C]c-Src (251-533) (GHM at N-terminal) (c-Src "ES1")

<400> SEQUENCE: 9

Gly His Met Gln Thr Gln Gly Leu Ala Lys Asp Ala Trp Glu Ile Pro  
 1 5 10 15  
 Arg Glu Ser Leu Arg Leu Glu Val Lys Leu Gly Gln Gly Cys Phe Gly  
 20 25 30  
 Glu Val Trp Met Gly Thr Trp Asn Gly Thr Thr Arg Val Ala Ile Lys  
 35 40 45  
 Thr Leu Lys Pro Gly Thr Met Ser Pro Glu Ala Phe Leu Gln Glu Ala  
 50 55 60  
 Gln Val Met Lys Lys Leu Arg His Glu Lys Leu Val Gln Leu Tyr Ala  
 65 70 75 80  
 Val Val Ser Glu Glu Pro Ile Tyr Ile Val Cys Glu Tyr Met Ser Lys  
 85 90 95  
 Gly Ser Leu Leu Asp Phe Leu Lys Gly Glu Met Gly Lys Tyr Leu Arg  
 100 105 110  
 Leu Pro Gln Leu Val Asp Met Ala Ala Gln Ile Ala Ser Gly Met Ala  
 115 120 125  
 Tyr Val Glu Arg Met Asn Tyr Val His Arg Asp Leu Arg Ala Ala Asn

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130	135	140
Ile Leu Val Gly Glu Asn	Leu Val Cys Lys Val	Ala Asp Phe Gly Leu
145	150	155
Ala Arg Leu Ile Glu Asp Asn	Glu Tyr Thr Ala Arg	Gln Gly Ala Lys
165	170	175
Phe Pro Ile Lys Trp Thr	Ala Pro Glu Ala Ala	Leu Tyr Gly Arg Phe
180	185	190
Thr Ile Lys Ser Asp Val	Trp Ser Phe Gly Ile	Leu Leu Thr Glu Leu
195	200	205
Thr Thr Lys Gly Arg Val	Pro Tyr Pro Gly Met	Val Asn Arg Glu Val
210	215	220
Leu Asp Gln Val Glu Arg	Gly Tyr Arg Met	Pro Cys Pro Pro Glu Cys
225	230	235
Pro Glu Ser Leu His Asp	Leu Met Cys Gln Cys	Trp Arg Lys Asp Pro
245	250	255
Glu Glu Arg Pro Thr Phe	Glu Tyr Leu Gln Ala	Phe Leu Glu Asp Tyr
260	265	270
Phe Thr Ser Thr Glu Pro	Gln Tyr Gln Pro Gly	Glu Asn Leu
275	280	285

<210> SEQ ID NO 10  
 <211> LENGTH: 283  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: synthetic Gallus gallus proto-oncogene [T338X,  
 V323X]c-Src (251-533)  
 <220> FEATURE:  
 <221> NAME/KEY: VARIANT  
 <222> LOCATION: (73)..(88)  
 <223> OTHER INFORMATION: Xaa = any naturally occurring amino acid

<400> SEQUENCE: 10

Gln Thr Gln Gly Leu Ala	Lys Asp Ala Trp Glu Ile	Pro Arg Glu Ser
1	5	10
Leu Arg Leu Glu Val Lys	Leu Gly Gln Gly Cys Phe	Gly Glu Val Trp
20	25	30
Met Gly Thr Trp Asn Gly	Thr Thr Arg Val Ala	Ile Lys Thr Leu Lys
35	40	45
Pro Gly Thr Met Ser Pro	Glu Ala Phe Leu Gln	Glu Ala Gln Val Met
50	55	60
Lys Lys Leu Arg His Glu	Lys Leu Xaa Gln Leu	Tyr Ala Val Val Ser
65	70	75
Glu Glu Pro Ile Tyr Ile	Val Xaa Glu Tyr Met	Ser Lys Gly Ser Leu
85	90	95
Leu Asp Phe Leu Lys Gly	Glu Met Gly Lys Tyr	Leu Arg Leu Pro Gln
100	105	110
Leu Val Asp Met Ala Ala	Gln Ile Ala Ser Gly	Met Ala Tyr Val Glu
115	120	125
Arg Met Asn Tyr Val His	Arg Asp Leu Arg Ala	Ala Asn Ile Leu Val
130	135	140
Gly Glu Asn Leu Val Cys	Lys Val Ala Asp Phe	Gly Leu Ala Arg Leu
145	150	155
Ile Glu Asp Asn Glu Tyr	Thr Ala Arg Gln Gly	Ala Lys Phe Pro Ile
165	170	175
Lys Trp Thr Ala Pro Glu	Ala Ala Leu Tyr Gly	Arg Phe Thr Ile Lys
180	185	190

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Ser Asp Val Trp Ser Phe Gly Ile Leu Leu Thr Glu Leu Thr Thr Lys  
 195 200 205  
 Gly Arg Val Pro Tyr Pro Gly Met Val Asn Arg Glu Val Leu Asp Gln  
 210 215 220  
 Val Glu Arg Gly Tyr Arg Met Pro Cys Pro Pro Glu Cys Pro Glu Ser  
 225 230 235 240  
 Leu His Asp Leu Met Cys Gln Cys Trp Arg Lys Asp Pro Glu Glu Arg  
 245 250 255  
 Pro Thr Phe Glu Tyr Leu Gln Ala Phe Leu Glu Asp Tyr Phe Thr Ser  
 260 265 270  
 Thr Glu Pro Gln Tyr Gln Pro Gly Glu Asn Leu  
 275 280

<210> SEQ ID NO 11  
 <211> LENGTH: 286  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: synthetic Gallus gallus proto-oncogene  
 GHM-[T338X, V323X]c-Src (251-533) (GHM at N-terminal)  
 <220> FEATURE:  
 <221> NAME/KEY: VARIANT  
 <222> LOCATION: (76)..(91)  
 <223> OTHER INFORMATION: Xaa = any naturally occurring amino acid

<400> SEQUENCE: 11

Gly His Met Gln Thr Gln Gly Leu Ala Lys Asp Ala Trp Glu Ile Pro  
 1 5 10 15  
 Arg Glu Ser Leu Arg Leu Glu Val Lys Leu Gly Gln Gly Cys Phe Gly  
 20 25 30  
 Glu Val Trp Met Gly Thr Trp Asn Gly Thr Thr Arg Val Ala Ile Lys  
 35 40 45  
 Thr Leu Lys Pro Gly Thr Met Ser Pro Glu Ala Phe Leu Gln Glu Ala  
 50 55 60  
 Gln Val Met Lys Lys Leu Arg His Glu Lys Leu Xaa Gln Leu Tyr Ala  
 65 70 75 80  
 Val Val Ser Glu Glu Pro Ile Tyr Ile Val Xaa Glu Tyr Met Ser Lys  
 85 90 95  
 Gly Ser Leu Leu Asp Phe Leu Lys Gly Glu Met Gly Lys Tyr Leu Arg  
 100 105 110  
 Leu Pro Gln Leu Val Asp Met Ala Ala Gln Ile Ala Ser Gly Met Ala  
 115 120 125  
 Tyr Val Glu Arg Met Asn Tyr Val His Arg Asp Leu Arg Ala Ala Asn  
 130 135 140  
 Ile Leu Val Gly Glu Asn Leu Val Cys Lys Val Ala Asp Phe Gly Leu  
 145 150 155 160  
 Ala Arg Leu Ile Glu Asp Asn Glu Tyr Thr Ala Arg Gln Gly Ala Lys  
 165 170 175  
 Phe Pro Ile Lys Trp Thr Ala Pro Glu Ala Ala Leu Tyr Gly Arg Phe  
 180 185 190  
 Thr Ile Lys Ser Asp Val Trp Ser Phe Gly Ile Leu Leu Thr Glu Leu  
 195 200 205  
 Thr Thr Lys Gly Arg Val Pro Tyr Pro Gly Met Val Asn Arg Glu Val  
 210 215 220  
 Leu Asp Gln Val Glu Arg Gly Tyr Arg Met Pro Cys Pro Pro Glu Cys  
 225 230 235 240

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Pro Glu Ser Leu His Asp Leu Met Cys Gln Cys Trp Arg Lys Asp Pro  
245 250 255

Glu Glu Arg Pro Thr Phe Glu Tyr Leu Gln Ala Phe Leu Glu Asp Tyr  
260 265 270

Phe Thr Ser Thr Glu Pro Gln Tyr Gln Pro Gly Glu Asn Leu  
275 280 285

&lt;210&gt; SEQ ID NO 12

&lt;211&gt; LENGTH: 283

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: synthetic Gallus gallus proto-oncogene [T338C, V323X]c-Src (251-533)

&lt;220&gt; FEATURE:

&lt;221&gt; NAME/KEY: VARIANT

&lt;222&gt; LOCATION: (73)..(73)

&lt;223&gt; OTHER INFORMATION: Xaa = any naturally occurring amino acid

&lt;400&gt; SEQUENCE: 12

Gln Thr Gln Gly Leu Ala Lys Asp Ala Trp Glu Ile Pro Arg Glu Ser  
1 5 10 15

Leu Arg Leu Glu Val Lys Leu Gly Gln Gly Cys Phe Gly Glu Val Trp  
20 25 30

Met Gly Thr Trp Asn Gly Thr Thr Arg Val Ala Ile Lys Thr Leu Lys  
35 40 45

Pro Gly Thr Met Ser Pro Glu Ala Phe Leu Gln Glu Ala Gln Val Met  
50 55 60

Lys Lys Leu Arg His Glu Lys Leu Xaa Gln Leu Tyr Ala Val Val Ser  
65 70 75 80

Glu Glu Pro Ile Tyr Ile Val Cys Glu Tyr Met Ser Lys Gly Ser Leu  
85 90 95

Leu Asp Phe Leu Lys Gly Glu Met Gly Lys Tyr Leu Arg Leu Pro Gln  
100 105 110

Leu Val Asp Met Ala Ala Gln Ile Ala Ser Gly Met Ala Tyr Val Glu  
115 120 125

Arg Met Asn Tyr Val His Arg Asp Leu Arg Ala Ala Asn Ile Leu Val  
130 135 140

Gly Glu Asn Leu Val Cys Lys Val Ala Asp Phe Gly Leu Ala Arg Leu  
145 150 155 160

Ile Glu Asp Asn Glu Tyr Thr Ala Arg Gln Gly Ala Lys Phe Pro Ile  
165 170 175

Lys Trp Thr Ala Pro Glu Ala Ala Leu Tyr Gly Arg Phe Thr Ile Lys  
180 185 190

Ser Asp Val Trp Ser Phe Gly Ile Leu Leu Thr Glu Leu Thr Thr Lys  
195 200 205

Gly Arg Val Pro Tyr Pro Gly Met Val Asn Arg Glu Val Leu Asp Gln  
210 215 220

Val Glu Arg Gly Tyr Arg Met Pro Cys Pro Pro Glu Cys Pro Glu Ser  
225 230 235 240

Leu His Asp Leu Met Cys Gln Cys Trp Arg Lys Asp Pro Glu Glu Arg  
245 250 255

Pro Thr Phe Glu Tyr Leu Gln Ala Phe Leu Glu Asp Tyr Phe Thr Ser  
260 265 270

Thr Glu Pro Gln Tyr Gln Pro Gly Glu Asn Leu  
275 280

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<210> SEQ ID NO 13
<211> LENGTH: 286
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic Gallus gallus proto-oncogene
      GHM-[T338C, V323X]c-Src (251-533) (GHM at N-terminal)
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: (76)..(76)
<223> OTHER INFORMATION: Xaa = any naturally occurring amino acid

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<400> SEQUENCE: 13

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Gly His Met Gln Thr Gln Gly Leu Ala Lys Asp Ala Trp Glu Ile Pro
1          5          10          15

Arg Glu Ser Leu Arg Leu Glu Val Lys Leu Gly Gln Gly Cys Phe Gly
20        25        30

Glu Val Trp Met Gly Thr Trp Asn Gly Thr Thr Arg Val Ala Ile Lys
35        40        45

Thr Leu Lys Pro Gly Thr Met Ser Pro Glu Ala Phe Leu Gln Glu Ala
50        55        60

Gln Val Met Lys Lys Leu Arg His Glu Lys Leu Xaa Gln Leu Tyr Ala
65        70        75        80

Val Val Ser Glu Glu Pro Ile Tyr Ile Val Cys Glu Tyr Met Ser Lys
85        90        95

Gly Ser Leu Leu Asp Phe Leu Lys Gly Glu Met Gly Lys Tyr Leu Arg
100       105       110

Leu Pro Gln Leu Val Asp Met Ala Ala Gln Ile Ala Ser Gly Met Ala
115       120       125

Tyr Val Glu Arg Met Asn Tyr Val His Arg Asp Leu Arg Ala Ala Asn
130       135       140

Ile Leu Val Gly Glu Asn Leu Val Cys Lys Val Ala Asp Phe Gly Leu
145       150       155       160

Ala Arg Leu Ile Glu Asp Asn Glu Tyr Thr Ala Arg Gln Gly Ala Lys
165       170       175

Phe Pro Ile Lys Trp Thr Ala Pro Glu Ala Ala Leu Tyr Gly Arg Phe
180       185       190

Thr Ile Lys Ser Asp Val Trp Ser Phe Gly Ile Leu Leu Thr Glu Leu
195       200       205

Thr Thr Lys Gly Arg Val Pro Tyr Pro Gly Met Val Asn Arg Glu Val
210       215       220

Leu Asp Gln Val Glu Arg Gly Tyr Arg Met Pro Cys Pro Pro Glu Cys
225       230       235       240

Pro Glu Ser Leu His Asp Leu Met Cys Gln Cys Trp Arg Lys Asp Pro
245       250       255

Glu Glu Arg Pro Thr Phe Glu Tyr Leu Gln Ala Phe Leu Glu Asp Tyr
260       265       270

Phe Thr Ser Thr Glu Pro Gln Tyr Gln Pro Gly Glu Asn Leu
275       280       285

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<210> SEQ ID NO 14
<211> LENGTH: 283
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic Gallus gallus proto-oncogene [T338C,
      V323A]c-Src (251-533) (c-Src "ES2")
<400> SEQUENCE: 14

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Gln Thr Gln Gly Leu Ala Lys Asp Ala Trp Glu Ile Pro Arg Glu Ser  
 1 5 10 15  
 Leu Arg Leu Glu Val Lys Leu Gly Gln Gly Cys Phe Gly Glu Val Trp  
 20 25 30  
 Met Gly Thr Trp Asn Gly Thr Thr Arg Val Ala Ile Lys Thr Leu Lys  
 35 40 45  
 Pro Gly Thr Met Ser Pro Glu Ala Phe Leu Gln Glu Ala Gln Val Met  
 50 55 60  
 Lys Lys Leu Arg His Glu Lys Leu Ala Gln Leu Tyr Ala Val Val Ser  
 65 70 75 80  
 Glu Glu Pro Ile Tyr Ile Val Cys Glu Tyr Met Ser Lys Gly Ser Leu  
 85 90 95  
 Leu Asp Phe Leu Lys Gly Glu Met Gly Lys Tyr Leu Arg Leu Pro Gln  
 100 105 110  
 Leu Val Asp Met Ala Ala Gln Ile Ala Ser Gly Met Ala Tyr Val Glu  
 115 120 125  
 Arg Met Asn Tyr Val His Arg Asp Leu Arg Ala Ala Asn Ile Leu Val  
 130 135 140  
 Gly Glu Asn Leu Val Cys Lys Val Ala Asp Phe Gly Leu Ala Arg Leu  
 145 150 155 160  
 Ile Glu Asp Asn Glu Tyr Thr Ala Arg Gln Gly Ala Lys Phe Pro Ile  
 165 170 175  
 Lys Trp Thr Ala Pro Glu Ala Ala Leu Tyr Gly Arg Phe Thr Ile Lys  
 180 185 190  
 Ser Asp Val Trp Ser Phe Gly Ile Leu Leu Thr Glu Leu Thr Thr Lys  
 195 200 205  
 Gly Arg Val Pro Tyr Pro Gly Met Val Asn Arg Glu Val Leu Asp Gln  
 210 215 220  
 Val Glu Arg Gly Tyr Arg Met Pro Cys Pro Pro Glu Cys Pro Glu Ser  
 225 230 235 240  
 Leu His Asp Leu Met Cys Gln Cys Trp Arg Lys Asp Pro Glu Glu Arg  
 245 250 255  
 Pro Thr Phe Glu Tyr Leu Gln Ala Phe Leu Glu Asp Tyr Phe Thr Ser  
 260 265 270  
 Thr Glu Pro Gln Tyr Gln Pro Gly Glu Asn Leu  
 275 280

<210> SEQ ID NO 15  
 <211> LENGTH: 286  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: synthetic Gallus gallus proto-oncogene  
 GHM-[T338C, V323A]c-Src (251-533) (GHM at N-terminal) (c-Src  
 "ES2")

<400> SEQUENCE: 15

Gly His Met Gln Thr Gln Gly Leu Ala Lys Asp Ala Trp Glu Ile Pro  
 1 5 10 15  
 Arg Glu Ser Leu Arg Leu Glu Val Lys Leu Gly Gln Gly Cys Phe Gly  
 20 25 30  
 Glu Val Trp Met Gly Thr Trp Asn Gly Thr Thr Arg Val Ala Ile Lys  
 35 40 45  
 Thr Leu Lys Pro Gly Thr Met Ser Pro Glu Ala Phe Leu Gln Glu Ala  
 50 55 60  
 Gln Val Met Lys Lys Leu Arg His Glu Lys Leu Ala Gln Leu Tyr Ala

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65	70	75	80
Val Val Ser Glu Glu Pro Ile Tyr Ile Val Cys Glu Tyr Met Ser Lys	85	90	95
Gly Ser Leu Leu Asp Phe Leu Lys Gly Glu Met Gly Lys Tyr Leu Arg	100	105	110
Leu Pro Gln Leu Val Asp Met Ala Ala Gln Ile Ala Ser Gly Met Ala	115	120	125
Tyr Val Glu Arg Met Asn Tyr Val His Arg Asp Leu Arg Ala Ala Asn	130	135	140
Ile Leu Val Gly Glu Asn Leu Val Cys Lys Val Ala Asp Phe Gly Leu	145	150	155
Ala Arg Leu Ile Glu Asp Asn Glu Tyr Thr Ala Arg Gln Gly Ala Lys	165	170	175
Phe Pro Ile Lys Trp Thr Ala Pro Glu Ala Ala Leu Tyr Gly Arg Phe	180	185	190
Thr Ile Lys Ser Asp Val Trp Ser Phe Gly Ile Leu Leu Thr Glu Leu	195	200	205
Thr Thr Lys Gly Arg Val Pro Tyr Pro Gly Met Val Asn Arg Glu Val	210	215	220
Leu Asp Gln Val Glu Arg Gly Tyr Arg Met Pro Cys Pro Pro Glu Cys	225	230	235
Pro Glu Ser Leu His Asp Leu Met Cys Gln Cys Trp Arg Lys Asp Pro	245	250	255
Glu Glu Arg Pro Thr Phe Glu Tyr Leu Gln Ala Phe Leu Glu Asp Tyr	260	265	270
Phe Thr Ser Thr Glu Pro Gln Tyr Gln Pro Gly Glu Asn Leu	275	280	285

&lt;210&gt; SEQ ID NO 16

&lt;211&gt; LENGTH: 283

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial sequence

&lt;220&gt; FEATURE:

<223> OTHER INFORMATION: synthetic Gallus gallus proto-oncogene [T338C, V323S]c-*Src* (251-533) (c-*Src* "ES3")

&lt;400&gt; SEQUENCE: 16

Gln Thr Gln Gly Leu Ala Lys Asp Ala Trp Glu Ile Pro Arg Glu Ser	1	5	10	15
Leu Arg Leu Glu Val Lys Leu Gly Gln Gly Cys Phe Gly Glu Val Trp	20	25	30	
Met Gly Thr Trp Asn Gly Thr Thr Arg Val Ala Ile Lys Thr Leu Lys	35	40	45	
Pro Gly Thr Met Ser Pro Glu Ala Phe Leu Gln Glu Ala Gln Val Met	50	55	60	
Lys Lys Leu Arg His Glu Lys Leu Ser Gln Leu Tyr Ala Val Val Ser	65	70	75	80
Glu Glu Pro Ile Tyr Ile Val Cys Glu Tyr Met Ser Lys Gly Ser Leu	85	90	95	
Leu Asp Phe Leu Lys Gly Glu Met Gly Lys Tyr Leu Arg Leu Pro Gln	100	105	110	
Leu Val Asp Met Ala Ala Gln Ile Ala Ser Gly Met Ala Tyr Val Glu	115	120	125	
Arg Met Asn Tyr Val His Arg Asp Leu Arg Ala Ala Asn Ile Leu Val	130	135	140	

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Gly Glu Asn Leu Val Cys Lys Val Ala Asp Phe Gly Leu Ala Arg Leu  
 145 150 155 160  
 Ile Glu Asp Asn Glu Tyr Thr Ala Arg Gln Gly Ala Lys Phe Pro Ile  
 165 170 175  
 Lys Trp Thr Ala Pro Glu Ala Ala Leu Tyr Gly Arg Phe Thr Ile Lys  
 180 185 190  
 Ser Asp Val Trp Ser Phe Gly Ile Leu Leu Thr Glu Leu Thr Thr Lys  
 195 200 205  
 Gly Arg Val Pro Tyr Pro Gly Met Val Asn Arg Glu Val Leu Asp Gln  
 210 215 220  
 Val Glu Arg Gly Tyr Arg Met Pro Cys Pro Pro Glu Cys Pro Glu Ser  
 225 230 235 240  
 Leu His Asp Leu Met Cys Gln Cys Trp Arg Lys Asp Pro Glu Glu Arg  
 245 250 255  
 Pro Thr Phe Glu Tyr Leu Gln Ala Phe Leu Glu Asp Tyr Phe Thr Ser  
 260 265 270  
 Thr Glu Pro Gln Tyr Gln Pro Gly Glu Asn Leu  
 275 280

<210> SEQ ID NO 17  
 <211> LENGTH: 286  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: synthetic Gallus gallus proto-oncogene  
 GHM-[T338C, V323S]c-Src (251-533) (GHM at N-terminal) (c-Src  
 "ES3")

<400> SEQUENCE: 17

Gly His Met Gln Thr Gln Gly Leu Ala Lys Asp Ala Trp Glu Ile Pro  
 1 5 10 15  
 Arg Glu Ser Leu Arg Leu Glu Val Lys Leu Gly Gln Gly Cys Phe Gly  
 20 25 30  
 Glu Val Trp Met Gly Thr Trp Asn Gly Thr Thr Arg Val Ala Ile Lys  
 35 40 45  
 Thr Leu Lys Pro Gly Thr Met Ser Pro Glu Ala Phe Leu Gln Glu Ala  
 50 55 60  
 Gln Val Met Lys Lys Leu Arg His Glu Lys Leu Ser Gln Leu Tyr Ala  
 65 70 75 80  
 Val Val Ser Glu Glu Pro Ile Tyr Ile Val Cys Glu Tyr Met Ser Lys  
 85 90 95  
 Gly Ser Leu Leu Asp Phe Leu Lys Gly Glu Met Gly Lys Tyr Leu Arg  
 100 105 110  
 Leu Pro Gln Leu Val Asp Met Ala Ala Gln Ile Ala Ser Gly Met Ala  
 115 120 125  
 Tyr Val Glu Arg Met Asn Tyr Val His Arg Asp Leu Arg Ala Ala Asn  
 130 135 140  
 Ile Leu Val Gly Glu Asn Leu Val Cys Lys Val Ala Asp Phe Gly Leu  
 145 150 155 160  
 Ala Arg Leu Ile Glu Asp Asn Glu Tyr Thr Ala Arg Gln Gly Ala Lys  
 165 170 175  
 Phe Pro Ile Lys Trp Thr Ala Pro Glu Ala Ala Leu Tyr Gly Arg Phe  
 180 185 190  
 Thr Ile Lys Ser Asp Val Trp Ser Phe Gly Ile Leu Leu Thr Glu Leu  
 195 200 205  
 Thr Thr Lys Gly Arg Val Pro Tyr Pro Gly Met Val Asn Arg Glu Val

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210	215	220
Leu Asp Gln Val Glu Arg Gly Tyr Arg Met Pro Cys Pro Pro Glu Cys		
225	230	235 240
Pro Glu Ser Leu His Asp Leu Met Cys Gln Cys Trp Arg Lys Asp Pro		
	245	250 255
Glu Glu Arg Pro Thr Phe Glu Tyr Leu Gln Ala Phe Leu Glu Asp Tyr		
	260	265 270
Phe Thr Ser Thr Glu Pro Gln Tyr Gln Pro Gly Glu Asn Leu		
	275	280 285
<210> SEQ ID NO 18		
<211> LENGTH: 283		
<212> TYPE: PRT		
<213> ORGANISM: Artificial sequence		
<220> FEATURE:		
<223> OTHER INFORMATION: synthetic Gallus gallus proto-oncogene [T338C, V323D]c-Src (251-533) (c-Src "ES4")		
<400> SEQUENCE: 18		
Gln Thr Gln Gly Leu Ala Lys Asp Ala Trp Glu Ile Pro Arg Glu Ser		
1	5	10 15
Leu Arg Leu Glu Val Lys Leu Gly Gln Gly Cys Phe Gly Glu Val Trp		
	20	25 30
Met Gly Thr Trp Asn Gly Thr Thr Arg Val Ala Ile Lys Thr Leu Lys		
	35	40 45
Pro Gly Thr Met Ser Pro Glu Ala Phe Leu Gln Glu Ala Gln Val Met		
	50	55 60
Lys Lys Leu Arg His Glu Lys Leu Asp Gln Leu Tyr Ala Val Val Ser		
65	70	75 80
Glu Glu Pro Ile Tyr Ile Val Cys Glu Tyr Met Ser Lys Gly Ser Leu		
	85	90 95
Leu Asp Phe Leu Lys Gly Glu Met Gly Lys Tyr Leu Arg Leu Pro Gln		
	100	105 110
Leu Val Asp Met Ala Ala Gln Ile Ala Ser Gly Met Ala Tyr Val Glu		
	115	120 125
Arg Met Asn Tyr Val His Arg Asp Leu Arg Ala Ala Asn Ile Leu Val		
	130	135 140
Gly Glu Asn Leu Val Cys Lys Val Ala Asp Phe Gly Leu Ala Arg Leu		
145	150	155 160
Ile Glu Asp Asn Glu Tyr Thr Ala Arg Gln Gly Ala Lys Phe Pro Ile		
	165	170 175
Lys Trp Thr Ala Pro Glu Ala Ala Leu Tyr Gly Arg Phe Thr Ile Lys		
	180	185 190
Ser Asp Val Trp Ser Phe Gly Ile Leu Leu Thr Glu Leu Thr Thr Lys		
	195	200 205
Gly Arg Val Pro Tyr Pro Gly Met Val Asn Arg Glu Val Leu Asp Gln		
	210	215 220
Val Glu Arg Gly Tyr Arg Met Pro Cys Pro Pro Glu Cys Pro Glu Ser		
225	230	235 240
Leu His Asp Leu Met Cys Gln Cys Trp Arg Lys Asp Pro Glu Glu Arg		
	245	250 255
Pro Thr Phe Glu Tyr Leu Gln Ala Phe Leu Glu Asp Tyr Phe Thr Ser		
	260	265 270
Thr Glu Pro Gln Tyr Gln Pro Gly Glu Asn Leu		
	275	280

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<210> SEQ ID NO 19  
 <211> LENGTH: 286  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: synthetic Gallus gallus proto-oncogene  
 GHM-[T338C, V323D]c-Src (251-533) (GHM at N-terminal) (c-Src  
 "ES4")

<400> SEQUENCE: 19

Gly His Met Gln Thr Gln Gly Leu Ala Lys Asp Ala Trp Glu Ile Pro  
 1 5 10 15  
 Arg Glu Ser Leu Arg Leu Glu Val Lys Leu Gly Gln Gly Cys Phe Gly  
 20 25 30  
 Glu Val Trp Met Gly Thr Trp Asn Gly Thr Thr Arg Val Ala Ile Lys  
 35 40 45  
 Thr Leu Lys Pro Gly Thr Met Ser Pro Glu Ala Phe Leu Gln Glu Ala  
 50 55 60  
 Gln Val Met Lys Lys Leu Arg His Glu Lys Leu Asp Gln Leu Tyr Ala  
 65 70 75 80  
 Val Val Ser Glu Glu Pro Ile Tyr Ile Val Cys Glu Tyr Met Ser Lys  
 85 90 95  
 Gly Ser Leu Leu Asp Phe Leu Lys Gly Glu Met Gly Lys Tyr Leu Arg  
 100 105 110  
 Leu Pro Gln Leu Val Asp Met Ala Ala Gln Ile Ala Ser Gly Met Ala  
 115 120 125  
 Tyr Val Glu Arg Met Asn Tyr Val His Arg Asp Leu Arg Ala Ala Asn  
 130 135 140  
 Ile Leu Val Gly Glu Asn Leu Val Cys Lys Val Ala Asp Phe Gly Leu  
 145 150 155 160  
 Ala Arg Leu Ile Glu Asp Asn Glu Tyr Thr Ala Arg Gln Gly Ala Lys  
 165 170 175  
 Phe Pro Ile Lys Trp Thr Ala Pro Glu Ala Ala Leu Tyr Gly Arg Phe  
 180 185 190  
 Thr Ile Lys Ser Asp Val Trp Ser Phe Gly Ile Leu Leu Thr Glu Leu  
 195 200 205  
 Thr Thr Lys Gly Arg Val Pro Tyr Pro Gly Met Val Asn Arg Glu Val  
 210 215 220  
 Leu Asp Gln Val Glu Arg Gly Tyr Arg Met Pro Cys Pro Pro Glu Cys  
 225 230 235 240  
 Pro Glu Ser Leu His Asp Leu Met Cys Gln Cys Trp Arg Lys Asp Pro  
 245 250 255  
 Glu Glu Arg Pro Thr Phe Glu Tyr Leu Gln Ala Phe Leu Glu Asp Tyr  
 260 265 270  
 Phe Thr Ser Thr Glu Pro Gln Tyr Gln Pro Gly Glu Asn Leu  
 275 280 285

<210> SEQ ID NO 20  
 <211> LENGTH: 283  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: synthetic Gallus gallus proto-oncogene [T338C,  
 V323E]c-Src (251-533) (c-Src "ES5")

<400> SEQUENCE: 20

Gln Thr Gln Gly Leu Ala Lys Asp Ala Trp Glu Ile Pro Arg Glu Ser  
 1 5 10 15

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Leu Arg Leu Glu Val Lys Leu Gly Gln Gly Cys Phe Gly Glu Val Trp  
                   20                                  25                                  30  
 Met Gly Thr Trp Asn Gly Thr Thr Arg Val Ala Ile Lys Thr Leu Lys  
                   35                                  40                                  45  
 Pro Gly Thr Met Ser Pro Glu Ala Phe Leu Gln Glu Ala Gln Val Met  
                   50                                  55                                  60  
 Lys Lys Leu Arg His Glu Lys Leu Glu Gln Leu Tyr Ala Val Val Ser  
                   65                                  70                                  75                                  80  
 Glu Glu Pro Ile Tyr Ile Val Cys Glu Tyr Met Ser Lys Gly Ser Leu  
                   85                                  90                                  95  
 Leu Asp Phe Leu Lys Gly Glu Met Gly Lys Tyr Leu Arg Leu Pro Gln  
                   100                                  105                                  110  
 Leu Val Asp Met Ala Ala Gln Ile Ala Ser Gly Met Ala Tyr Val Glu  
                   115                                  120                                  125  
 Arg Met Asn Tyr Val His Arg Asp Leu Arg Ala Ala Asn Ile Leu Val  
                   130                                  135                                  140  
 Gly Glu Asn Leu Val Cys Lys Val Ala Asp Phe Gly Leu Ala Arg Leu  
                   145                                  150                                  155                                  160  
 Ile Glu Asp Asn Glu Tyr Thr Ala Arg Gln Gly Ala Lys Phe Pro Ile  
                   165                                  170                                  175  
 Lys Trp Thr Ala Pro Glu Ala Ala Leu Tyr Gly Arg Phe Thr Ile Lys  
                   180                                  185                                  190  
 Ser Asp Val Trp Ser Phe Gly Ile Leu Leu Thr Glu Leu Thr Thr Lys  
                   195                                  200                                  205  
 Gly Arg Val Pro Tyr Pro Gly Met Val Asn Arg Glu Val Leu Asp Gln  
                   210                                  215                                  220  
 Val Glu Arg Gly Tyr Arg Met Pro Cys Pro Pro Glu Cys Pro Glu Ser  
                   225                                  230                                  235                                  240  
 Leu His Asp Leu Met Cys Gln Cys Trp Arg Lys Asp Pro Glu Glu Arg  
                   245                                  250                                  255  
 Pro Thr Phe Glu Tyr Leu Gln Ala Phe Leu Glu Asp Tyr Phe Thr Ser  
                   260                                  265                                  270  
 Thr Glu Pro Gln Tyr Gln Pro Gly Glu Asn Leu  
                   275                                  280

<210> SEQ ID NO 21  
 <211> LENGTH: 286  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: synthetic Gallus gallus proto-oncogene  
                                   GHM-[T338C, V323E]c-Src (251-533) (GHM at N-terminal) (c-Src  
                                   "ES5")

<400> SEQUENCE: 21

Gly His Met Gln Thr Gln Gly Leu Ala Lys Asp Ala Trp Glu Ile Pro  
 1                                  5                                  10                                  15  
 Arg Glu Ser Leu Arg Leu Glu Val Lys Leu Gly Gln Gly Cys Phe Gly  
                   20                                  25                                  30  
 Glu Val Trp Met Gly Thr Trp Asn Gly Thr Thr Arg Val Ala Ile Lys  
                   35                                  40                                  45  
 Thr Leu Lys Pro Gly Thr Met Ser Pro Glu Ala Phe Leu Gln Glu Ala  
                   50                                  55                                  60  
 Gln Val Met Lys Lys Leu Arg His Glu Lys Leu Glu Gln Leu Tyr Ala  
                   65                                  70                                  75                                  80

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Val	Val	Ser	Glu	Glu	Pro	Ile	Tyr	Ile	Val	Cys	Glu	Tyr	Met	Ser	Lys
			85					90						95	
Gly	Ser	Leu	Leu	Asp	Phe	Leu	Lys	Gly	Glu	Met	Gly	Lys	Tyr	Leu	Arg
		100					105					110			
Leu	Pro	Gln	Leu	Val	Asp	Met	Ala	Ala	Gln	Ile	Ala	Ser	Gly	Met	Ala
		115					120				125				
Tyr	Val	Glu	Arg	Met	Asn	Tyr	Val	His	Arg	Asp	Leu	Arg	Ala	Ala	Asn
	130				135						140				
Ile	Leu	Val	Gly	Glu	Asn	Leu	Val	Cys	Lys	Val	Ala	Asp	Phe	Gly	Leu
145				150						155					160
Ala	Arg	Leu	Ile	Glu	Asp	Asn	Glu	Tyr	Thr	Ala	Arg	Gln	Gly	Ala	Lys
			165					170						175	
Phe	Pro	Ile	Lys	Trp	Thr	Ala	Pro	Glu	Ala	Ala	Leu	Tyr	Gly	Arg	Phe
		180					185						190		
Thr	Ile	Lys	Ser	Asp	Val	Trp	Ser	Phe	Gly	Ile	Leu	Leu	Thr	Glu	Leu
		195				200						205			
Thr	Thr	Lys	Gly	Arg	Val	Pro	Tyr	Pro	Gly	Met	Val	Asn	Arg	Glu	Val
	210				215						220				
Leu	Asp	Gln	Val	Glu	Arg	Gly	Tyr	Arg	Met	Pro	Cys	Pro	Pro	Glu	Cys
225				230						235					240
Pro	Glu	Ser	Leu	His	Asp	Leu	Met	Cys	Gln	Cys	Trp	Arg	Lys	Asp	Pro
			245					250						255	
Glu	Glu	Arg	Pro	Thr	Phe	Glu	Tyr	Leu	Gln	Ala	Phe	Leu	Glu	Asp	Tyr
			260					265					270		
Phe	Thr	Ser	Thr	Glu	Pro	Gln	Tyr	Gln	Pro	Gly	Glu	Asn	Leu		
		275				280						285			

&lt;210&gt; SEQ ID NO 22

&lt;211&gt; LENGTH: 283

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: synthetic Gallus gallus proto-oncogene [T338C, V323H]c-Src (251-533) (c-Src "ES6")

&lt;400&gt; SEQUENCE: 22

Gln	Thr	Gln	Gly	Leu	Ala	Lys	Asp	Ala	Trp	Glu	Ile	Pro	Arg	Glu	Ser
1				5					10					15	
Leu	Arg	Leu	Glu	Val	Lys	Leu	Gly	Gln	Gly	Cys	Phe	Gly	Glu	Val	Trp
		20					25						30		
Met	Gly	Thr	Trp	Asn	Gly	Thr	Thr	Arg	Val	Ala	Ile	Lys	Thr	Leu	Lys
	35				40							45			
Pro	Gly	Thr	Met	Ser	Pro	Glu	Ala	Phe	Leu	Gln	Glu	Ala	Gln	Val	Met
	50				55					60					
Lys	Lys	Leu	Arg	His	Glu	Lys	Leu	His	Gln	Leu	Tyr	Ala	Val	Val	Ser
65				70					75					80	
Glu	Glu	Pro	Ile	Tyr	Ile	Val	Cys	Glu	Tyr	Met	Ser	Lys	Gly	Ser	Leu
		85						90					95		
Leu	Asp	Phe	Leu	Lys	Gly	Glu	Met	Gly	Lys	Tyr	Leu	Arg	Leu	Pro	Gln
		100					105						110		
Leu	Val	Asp	Met	Ala	Ala	Gln	Ile	Ala	Ser	Gly	Met	Ala	Tyr	Val	Glu
		115				120						125			
Arg	Met	Asn	Tyr	Val	His	Arg	Asp	Leu	Arg	Ala	Ala	Asn	Ile	Leu	Val
	130				135							140			
Gly	Glu	Asn	Leu	Val	Cys	Lys	Val	Ala	Asp	Phe	Gly	Leu	Ala	Arg	Leu
145				150						155					160

Ile	Glu	Asp	Asn	Glu	Tyr	Thr	Ala	Arg	Gln	Gly	Ala	Lys	Phe	Thr	Ile	Pro	Ile
				165					170							175	
Lys	Trp	Thr	Ala	Pro	Glu	Ala	Ala	Leu	Tyr	Gly	Arg	Phe	Thr	Ile	Lys		
			180					185					190				
Ser	Asp	Val	Trp	Ser	Phe	Gly	Ile	Leu	Leu	Thr	Glu	Leu	Thr	Thr	Lys		
		195				200						205					
Gly	Arg	Val	Pro	Tyr	Pro	Gly	Met	Val	Asn	Arg	Glu	Val	Leu	Asp	Gln		
	210					215					220						
Val	Glu	Arg	Gly	Tyr	Arg	Met	Pro	Cys	Pro	Pro	Glu	Cys	Pro	Glu	Ser		
225				230					235						240		
Leu	His	Asp	Leu	Met	Cys	Gln	Cys	Trp	Arg	Lys	Asp	Pro	Glu	Glu	Arg		
			245						250					255			
Pro	Thr	Phe	Glu	Tyr	Leu	Gln	Ala	Phe	Leu	Glu	Asp	Tyr	Phe	Thr	Ser		
		260						265					270				
Thr	Glu	Pro	Gln	Tyr	Gln	Pro	Gly	Glu	Asn	Leu							
	275					280											
<210> SEQ ID NO 23																	
<211> LENGTH: 286																	
<212> TYPE: PRT																	
<213> ORGANISM: Artificial sequence																	
<220> FEATURE:																	
<223> OTHER INFORMATION: synthetic Gallus gallus proto-oncogene GHM-[T338C, V323H]c-Src (251-533) (GHM at N-terminal) (c-Src "ES6")																	
<400> SEQUENCE: 23																	
Gly	His	Met	Gln	Thr	Gln	Gly	Leu	Ala	Lys	Asp	Ala	Trp	Glu	Ile	Pro		
1				5					10					15			
Arg	Glu	Ser	Leu	Arg	Leu	Glu	Val	Lys	Leu	Gly	Gln	Gly	Cys	Phe	Gly		
			20					25					30				
Glu	Val	Trp	Met	Gly	Thr	Trp	Asn	Gly	Thr	Thr	Arg	Val	Ala	Ile	Lys		
		35					40					45					
Thr	Leu	Lys	Pro	Gly	Thr	Met	Ser	Pro	Glu	Ala	Phe	Leu	Gln	Glu	Ala		
	50					55					60						
Gln	Val	Met	Lys	Lys	Leu	Arg	His	Glu	Lys	Leu	His	Gln	Leu	Tyr	Ala		
65				70					75						80		
Val	Val	Ser	Glu	Glu	Pro	Ile	Tyr	Ile	Val	Cys	Glu	Tyr	Met	Ser	Lys		
			85						90				95				
Gly	Ser	Leu	Leu	Asp	Phe	Leu	Lys	Gly	Glu	Met	Gly	Lys	Tyr	Leu</			

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Leu Asp Gln Val Glu Arg Gly Tyr Arg Met Pro Cys Pro Pro Glu Cys  
 225 230 235 240  
 Pro Glu Ser Leu His Asp Leu Met Cys Gln Cys Trp Arg Lys Asp Pro  
 245 250 255  
 Glu Glu Arg Pro Thr Phe Glu Tyr Leu Gln Ala Phe Leu Glu Asp Tyr  
 260 265 270  
 Phe Thr Ser Thr Glu Pro Gln Tyr Gln Pro Gly Glu Asn Leu  
 275 280 285

<210> SEQ ID NO 24  
 <211> LENGTH: 570  
 <212> TYPE: PRT  
 <213> ORGANISM: Arabidopsis thaliana  
 <220> FEATURE:  
 <223> OTHER INFORMATION: hypothetical Ser-Thr protein kinase, kinase  
 with gatekeeper Cys

<400> SEQUENCE: 24

Met Ala Val Asp Val Lys Ser Val Leu Glu Phe Leu Arg Arg Asn Gly  
 1 5 10 15  
 Leu Thr Glu Ala Glu Ser Ala Leu Arg Asp Asp Ile Asn Glu Lys Asn  
 20 25 30  
 Lys Leu Ala Ser Phe Asp Phe Glu Lys Phe Leu Phe Pro Ile Pro Thr  
 35 40 45  
 Pro Ile Lys Ile Thr Ala Ser Ser Arg Pro Ser Asp Ser Gly Gly Asp  
 50 55 60  
 Gly Ser Asn Ser Lys Ser Ser Ser Ser Asp Asp Glu Phe Val Ser Leu  
 65 70 75 80  
 Asp Ser Ser Thr Ser Gly Phe Cys Ser Ser Ser Gly Phe Val Asn Pro  
 85 90 95  
 Tyr Gly Asp Ser Ser Ser Ser Ser Asp Gly Gln Ser Gln Phe Gly Thr  
 100 105 110  
 Ala Arg Thr Tyr Pro Glu Trp Ser Glu Phe Tyr Leu His Asn Glu Thr  
 115 120 125  
 Glu Asp Glu Asp Glu Phe Met Ser Pro Ala Phe Arg Glu Ser Asp Cys  
 130 135 140  
 Phe Ile Leu Pro Glu Asn Ala Glu Asp Lys Phe Ile Thr Asp Asn Gln  
 145 150 155 160  
 Phe Glu Asn Ser Leu Gly Val Tyr Asp Arg Ser Ser Ser Gln Gly Ser  
 165 170 175  
 Leu Thr Glu Ala Ser Leu Asp Tyr Leu Asp Lys Pro Phe Leu Leu Asp  
 180 185 190  
 Ile Gly Leu Glu Asp Lys Thr Asp Glu Leu Asp Leu Lys Thr Gly Asp  
 195 200 205  
 Gln Leu Asn Val Thr Asp Glu Glu Val Asp Val Val His Glu Val Glu  
 210 215 220  
 Asp Glu Tyr Glu Val Phe Asn Leu Arg Ile Ile His Trp Lys Asn Arg  
 225 230 235 240  
 Thr Gly Phe Glu Glu Asn Lys Asp Leu Pro Ile Val Ile Asn Thr Val  
 245 250 255  
 Ile Gly Gly Arg Tyr Tyr Ile Thr Glu Tyr Ile Gly Ser Ala Ala Phe  
 260 265 270  
 Ser Lys Val Val Gln Ala Gln Asp Leu His Asn Gly Val Asp Val Cys  
 275 280 285  
 Leu Lys Ile Ile Lys Asn Asp Lys Asp Phe Phe Asp Gln Ser Leu Asp  
 290 295 300

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Glu Ile Lys Leu Leu Lys His Val Asn Lys His Asp Pro Ala Asp Glu  
 305 310 315 320  
 His His Ile Leu Arg Leu Tyr Asp Tyr Phe Tyr His Gln Glu His Leu  
 325 330 335  
 Phe Ile Val Cys Glu Leu Leu Arg Ala Asn Leu Tyr Glu Phe Gln Lys  
 340 345 350  
 Phe Asn Gln Glu Ser Gly Gly Glu Pro Tyr Phe Asn Leu Ser Arg Leu  
 355 360 365  
 Gln Val Ile Thr Arg Gln Cys Leu Asp Ala Leu Val Phe Leu His Gly  
 370 375 380  
 Leu Gly Ile Ile His Cys Asp Leu Lys Pro Glu Asn Ile Leu Ile Lys  
 385 390 395 400  
 Ser Tyr Lys Arg Cys Ala Val Lys Ile Ile Asp Leu Gly Ser Ser Cys  
 405 410 415  
 Phe Arg Ser Asp Asn Leu Cys Leu Tyr Val Gln Ser Arg Ser Tyr Arg  
 420 425 430  
 Ala Pro Glu Val Ile Leu Gly Leu Pro Tyr Asp Glu Lys Ile Asp Leu  
 435 440 445  
 Trp Ser Leu Gly Cys Ile Leu Ala Glu Leu Cys Ser Gly Glu Val Leu  
 450 455 460  
 Phe Pro Asn Glu Ala Val Ala Met Ile Leu Ala Arg Ile Val Ala Val  
 465 470 475 480  
 Leu Gly Pro Ile Glu Thr Glu Met Leu Glu Lys Gly Gln Glu Thr His  
 485 490 495  
 Lys Tyr Phe Thr Lys Glu Tyr Asp Leu Tyr His Leu Asn Glu Glu Ser  
 500 505 510  
 Asn Glu Ile Glu Tyr Ile Ile Thr Glu Glu Ser Ser Leu Glu Glu Gln  
 515 520 525  
 Leu Gln Val Ser Asp Glu Leu Phe Leu Asp Phe Val Arg Thr Leu Leu  
 530 535 540  
 Asp Ile Asn Pro Leu Arg Arg Pro Thr Ala Leu Glu Ala Leu Asn His  
 545 550 555 560  
 Pro Trp Leu Ser Ser Ser Ser Ser Tyr Asn  
 565 570

<210> SEQ ID NO 25  
 <211> LENGTH: 276  
 <212> TYPE: PRT  
 <213> ORGANISM: Arabidopsis thaliana  
 <220> FEATURE:  
 <223> OTHER INFORMATION: hypothetical Ser-Thr protein kinase, kinase  
 with gatekeeper Cys

<400> SEQUENCE: 25

Asp Phe Phe Asp Gln Ser Leu Asp Glu Ile Lys Leu Leu Lys Tyr Val  
 1 5 10 15  
 Asn Lys His Asp Pro Ala Asp Lys Tyr His Leu Leu Arg Leu Tyr Asp  
 20 25 30  
 Tyr Phe Tyr Tyr Arg Glu His Leu Leu Ile Val Cys Glu Leu Leu Lys  
 35 40 45  
 Ala Asn Leu Tyr Glu Phe His Lys Phe Asn Arg Glu Ser Gly Gly Glu  
 50 55 60  
 Val Tyr Phe Thr Met Pro Arg Leu Gln Ser Ile Thr Ile Gln Cys Leu  
 65 70 75 80  
 Glu Ser Leu Gln Phe Leu His Gly Leu Gly Leu Ile His Cys Asp Leu

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85					90					95					
Lys	Pro	Glu	Asn	Ile	Leu	Val	Lys	Ser	Tyr	Ser	Arg	Cys	Glu	Ile	Lys
			100					105					110		
Val	Ile	Asp	Leu	Gly	Ser	Ser	Cys	Phe	Glu	Thr	Asp	His	Leu	Cys	Ser
		115					120					125			
Tyr	Val	Gln	Ser	Arg	Ser	Tyr	Arg	Ala	Pro	Glu	Val	Ile	Leu	Gly	Leu
		130					135					140			
Pro	Tyr	Asp	Lys	Lys	Ile	Asp	Val	Trp	Ser	Leu	Gly	Cys	Ile	Leu	Ala
				150								155			160
Glu	Leu	Cys	Thr	Gly	Asn	Val	Leu	Phe	Arg	Asn	Asp	Ser	Pro	Ala	Ser
				165					170					175	
Leu	Leu	Ala	Arg	Val	Met	Gly	Ile	Val	Gly	Ser	Phe	Asp	Asn	Glu	Met
			180					185					190		
Leu	Thr	Lys	Gly	Arg	Asp	Ser	His	Lys	Tyr	Phe	Thr	Lys	Asn	Arg	Met
		195						200					205		
Leu	Tyr	Glu	Arg	Asn	Gln	Glu	Ser	Asn	Arg	Leu	Glu	Tyr	Leu	Ile	Pro
		210					215					220			
Lys	Arg	Thr	Ser	Leu	Arg	His	Arg	Leu	Pro	Met	Gly	Asp	Gln	Gly	Phe
				230								235			240
Thr	Asp	Phe	Val	Ala	His	Leu	Leu	Glu	Ile	Asn	Pro	Lys	Lys	Arg	Pro
				245					250					255	
Ser	Ala	Ala	Glu	Ala	Leu	Lys	His	Pro	Trp	Leu	Ser	Tyr	Pro	Tyr	Glu
			260					265					270		
Pro	Ile	Ser	Ala												
			275												

<210> SEQ ID NO 26  
 <211> LENGTH: 617  
 <212> TYPE: PRT  
 <213> ORGANISM: Arabidopsis thaliana  
 <220> FEATURE:  
 <223> OTHER INFORMATION: putative serine/threonine protein kinase,  
 kinase with gatekeeper Cys

<400> SEQUENCE: 26

Met	Leu	Phe	Leu	Arg	Arg	Ile	Ala	Val	Val	Phe	Phe	Val	Phe	Thr	Ser
1				5					10					15	
Phe	Ser	Ala	Ala	Gln	Asn	Ser	Thr	Cys	Pro	Leu	Asp	Phe	Ser	Val	Leu
			20					25				30			
Glu	Pro	Phe	Arg	Arg	Pro	Lys	Pro	Asp	Gly	Ala	Thr	Thr	Cys	Gln	Tyr
		35				40						45			
Leu	Leu	Gln	Gly	Leu	Arg	Leu	Leu	Tyr	Ser	His	His	Leu	Arg	Gln	Thr
		50				55					60				
Gly	Ser	Phe	Leu	Pro	Pro	Pro	Glu	Ser	Ala	Ala	Ser	Cys	Trp	Ala	Ala
				70						75				80	
Leu	Gln	Ser	Ser	Val	Ala	Gly	Phe	Leu	Pro	Arg	Phe	Asp	Val	Arg	Ser
				85					90					95	
Thr	Cys	Gly	Phe	Gln	Thr	Pro	Trp	Ile	Ser	Gln	Gly	Cys	Met	Asp	Ile
		100						105					110		
Thr	Thr	Arg	Ser	Gln	Phe	Glu	Ser	Leu	Ile	Pro	Asn	Ser	Ser	Leu	Ala
		115						120					125		
Thr	Thr	Ala	Met	Arg	Cys	Asn	Thr	Ser	Leu	Glu	Ser	Asn	Thr	Pro	Cys
		130				135						140			
Ala	Ser	Cys	Thr	Gln	Ser	Leu	Ser	Ala	Phe	Gln	Pro	Tyr	Leu	Ser	Gly
				150						155					160

Pro	Ser	Leu	Gly	Asn	Val	Ser	Asp	Cys	Ala	Ser	Phe	Pro	Ser	Ile	Tyr
				165					170					175	
Ala	Ala	Ala	Phe	Ala	Asn	Ser	Leu	Gly	Pro	Thr	Asp	Lys	Gly	Thr	Ala
			180					185				190			
Lys	Cys	Leu	Phe	Gln	Leu	Asp	Leu	Ala	Ser	Pro	Thr	Ser	Ser	Gly	Ala
		195					200					205			
Asn	Lys	Val	Lys	Val	Leu	Val	Ser	Ser	Phe	Ser	Val	Leu	Leu	Val	Ala
		210				215					220				
Ser	Val	Leu	Val	Ile	Thr	Ala	Trp	Phe	Trp	Tyr	Cys	Arg	Arg	Lys	Lys
					230					235					240
Ser	Lys	Leu	Leu	Lys	Pro	Arg	Asp	Thr	Ser	Leu	Glu	Ala	Gly	Thr	Gln
				245				250						255	
Ser	Arg	Leu	Asp	Ser	Met	Ser	Glu	Ser	Thr	Thr	Leu	Val	Lys	Phe	Ser
			260					265					270		
Phe	Asp	Glu	Ile	Lys	Lys	Ala	Thr	Asn	Asn	Phe	Ser	Arg	His	Asn	Ile
							280					285			
Ile	Gly	Arg	Gly	Gly	Tyr	Gly	Asn	Val	Phe	Lys	Gly	Ala	Leu	Pro	Asp
		290				295					300				
Gly	Thr	Gln	Val	Ala	Phe	Lys	Arg	Phe	Lys	Asn	Cys	Ser	Ala	Gly	Gly
					310					315					320
Asp	Ala	Asn	Phe	Ala	His	Glu	Val	Glu	Val	Ile	Ala	Ser	Ile	Arg	His
				325					330					335	
Val	Asn	Leu	Leu	Ala	Leu	Arg	Gly	Tyr	Cys	Thr	Ala	Thr	Thr	Pro	Tyr
			340					345					350		
Glu	Gly	His	Gln	Arg	Ile	Ile	Val	Cys	Asp	Leu	Val	Ser	Asn	Gly	Ser
		355					360					365			
Leu	His	Asp	His	Leu	Phe	Gly	Asp	Leu	Glu	Ala	Gln	Leu	Ala	Trp	Pro
		370				375					380				
Leu	Arg	Gln	Arg	Ile	Ala	Leu	Gly	Met	Ala	Arg	Gly	Leu	Ala	Tyr	Leu
					390					395					400
His	Tyr	Gly	Ala	Gln	Pro	Ser	Ile	Ile	His	Arg	Asp	Ile	Lys	Ala	Ser
			405						410					415	
Asn	Ile	Leu	Leu	Asp	Glu	Arg	Phe	Glu	Ala	Lys	Val	Ala	Asp	Phe	Gly
			420					425					430		
Leu	Ala	Lys	Phe	Asn	Pro	Glu	Gly	Met	Thr	His	Met	Ser	Thr	Arg	Val
		435					440					445			
Ala	Gly	Thr	Met	Gly	Tyr	Val	Ala	Pro	Glu	Tyr	Ala	Leu	Tyr	Gly	Gln
		450				455					460				
Leu	Thr	Glu	Lys	Ser	Asp	Val	Tyr	Ser	Phe	Gly	Val	Val	Leu	Leu	Glu
					470					475					480
Leu	Leu	Ser	Arg	Arg	Lys	Ala	Ile	Val	Thr	Asp	Glu	Glu	Gly	Gln	Pro
				485					490					495	
Val	Ser	Val	Ala	Asp	Trp	Ala	Trp	Ser	Leu	Val	Arg	Glu	Gly	Gln	Thr
			500					505					510		
Leu	Asp	Val	Val	G											

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580	585	590
Lys Leu Thr Ser Pro Thr Gly	Tyr Gln Ala Phe Ser Phe Gly Gly Asp	
595	600	605
Gly Pro Ser Gly Asn Thr Asn Thr Thr		
610	615	
<210> SEQ ID NO 27		
<211> LENGTH: 369		
<212> TYPE: PRT		
<213> ORGANISM: Arabidopsis thaliana		
<220> FEATURE:		
<223> OTHER INFORMATION: protein kinase-like protein, kinase with gatekeeper Cys		
<400> SEQUENCE: 27		
Met Ala His Ile Ser Asp Ile Lys Leu Ile Arg Thr Asp Thr Thr Leu		
1	5	10
Asp Leu Ser Gln Lys Ala Glu Lys Gly Met Ile Trp Thr Met Gly Gly		
	20	25
Ala Ser Tyr Leu Tyr Tyr Asn Ala Tyr Asp His Gly Ser Leu Thr Cys		
	35	40
Arg Cys Gly Thr Leu Leu Ile Ala Ser Ser Gly Gly Lys Tyr Asn Pro		
	50	55
Ile Arg Thr Phe Ser Ser His Gln Ile Leu Glu Ala Thr Asn Asn Phe		
	65	70
Asp Trp Ser Tyr Ala Ile Gly Val Asp Arg Phe Val Trp Tyr Lys Gly		
	85	90
Thr Ile Glu Asn Arg Ala Val Leu Ile Lys Tyr Tyr Lys Gly Glu Pro		
	100	105
Phe Asn Phe Asp Pro Asp Asn Phe Tyr Arg Asp Ile Ala Val Ser Ser		
	115	120
Met Met Ser Ser His Lys Asn Val Leu Lys Leu Leu Gly Cys Cys Leu		
	130	135
Glu Phe Pro Arg Pro Val Leu Val Cys Glu Tyr Pro Glu Lys Gly Ala		
	145	150
Leu Ala Tyr Ile Gly Gly Ala Gly Glu Val Ile Lys Pro Leu Ala Trp		
	165	170
Ser Val Arg Leu Lys Ile Ala Lys Glu Ile Ala Asp Ala Val Thr Tyr		
	180	185
Leu His Thr Glu Phe Pro Arg Thr Ile Ile His Arg Asp Leu Lys Leu		
	195	200
Thr Asn Ile Phe Leu Asp Glu Asn Trp Thr Ala Lys Leu Ser Ser Phe		
	210	215
Ser Leu Ser Ile Pro Ile Pro Glu Gly Glu Leu Gly Val Glu Asp Ile		
	225	230
Val Cys Gly Thr Gln Gly Phe Gly Glu Pro His Tyr Met Val Thr Gly		
	245	250
Phe Val Thr Glu Asn Val Asp Ile Tyr Ser Phe Gly Phe Ile Met Leu		
	260	265
Ser Leu Leu Thr Gly Lys His Gly Phe Tyr Gln Glu Pro Ala Asn Gly		
	275	280
Asp Ser Tyr Asn Met Ile Leu Leu Pro Asp Tyr Val Glu Lys Cys Leu		
	290	295
Gly Arg Gly Pro Leu Ala Lys Leu Ile Asp Pro Ser Met Leu Asn Ser		
	305	310
		315
		320

Thr	Asp	Asp	Asp	Ile	Pro	Asp	His	Ser	Lys	Leu	Gln	Met	Glu	Ala	Phe	
				325					330						335	
Val	Asn	Leu	Ala	Leu	Arg	Cys	Val	Gly	Phe	Arg	Ser	Gly	Glu	Thr	Lys	
			340					345					350			
Leu	His	Met	Ile	Asp	Val	Ala	Lys	Glu	Leu	Lys	Arg	Ile	Gln	Lys	Gln	
		355					360					365				

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<210> SEQ ID NO 28
<211> LENGTH: 372
<212> TYPE: PRT
<213> ORGANISM: Arabidopsis thaliana
<220> FEATURE:
<223> OTHER INFORMATION: putative kinase-like protein, kinase with
gatekeeper Cys
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Met 1	Asp	Lys	Ile	Ile 5	Ile	Ser	Asn	Leu	Ser 10	Glu	Phe	Asp	His	Ser 15	Val
Val	Asp	Tyr	His 20	Lys	Ser	Ser	Leu	Leu 25	Leu	Cys	Lys	Ser	Gln 30	Ser	Phe
Glu	Leu	Ser 35	Pro	Ile	Glu	Met	Ser 40	Lys	Asn	Asn	Lys	Lys 45	Lys	Arg	Arg
Trp	Asp 50	Leu	Lys	Asn	Gly	Gly 55	Ile	Leu	Leu	Glu	Glu 60	Leu	Ile	Ala	Ser
Phe 65	Asp	Gly	Lys	Thr	Asn 70	Pro	Ile	Arg	Cys	Phe 75	Ser	Ser	Asp	Gln	Ile 80
Leu	Lys	Ala	Thr 85	Asp	Asn	Phe	Ser	Glu	Ser 90	Arg	Ile	Ile	Ser	Ser 95	Trp
Gly	Tyr	Phe	Ile 100	Trp	Tyr	Lys	Gly	Val 105	Ile	Glu	Glu	Arg	Gln 110	Val	Ser
Ile	Lys	Lys 115	Trp	Ser	Ser	Gln	Asn 120	Leu	Ser	Ser	Phe	Thr 125	Glu	Ala	Tyr
Arg	Asp 130	Ile	Ser	Val	Ser	Ser 135	Gln	Met	Ser	Gly	His 140	Lys	Asn	Ala	Leu
Lys 145	Leu	Ile	Gly	Cys	Cys 150	Leu	Glu	Phe	Asp	Leu 155	Pro	Ala	Leu	Val	Cys 160
Glu	Tyr	Thr	Glu	His 165	Gly	Pro	Leu	Asn	Arg	Asp	Gly	Gly	Leu	Ser 175	Ser
Gly	Val	Val	Leu 180	Pro	Trp	Lys	Val	Arg 185	Leu	Lys	Ile	Ala	Lys 190	Glu	Ile
Ala	Ser 195	Ser	Val	Thr	Tyr	Leu	His 200	Thr	Ala	Phe	Pro	Glu 205	Thr	Ile	Val
His 210	Arg	Asn	Ile	Asn	Pro	Thr 215	Asn	Ile	Phe	Ile	Asp 220	Glu	Asn	Trp	Thr
Ala 225	Lys	Leu	Ser	Asp	Phe 230	Trp	Phe	Cys	Val	Ala 235	Ile	Pro	Glu	Gly	Glu 240
Leu	Tyr	Val	Glu	Asp 245	Asp	Val	Lys	Gly	Val 250	Ile	Gly	Phe	Val	Asp 255	Pro
Asp	Tyr	Tyr	Trp 260	Thr	Met	Lys	Val	Thr 265	Glu	Lys	Val	Asp	Ile 270	Tyr	Ser
Phe	Gly 275	Val	Val	Met	Leu	Val	Leu 280	Leu	Ser	Gly	Arg	Ala 285	Ala	Val	Phe
Asn 290	Gly	Pro	Asp	Glu	Ala	Pro 295	Met	Ser	Leu	Asn	Asp 300	His	Val	Ser	Glu

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Val	Met	Glu	Lys	Gly	Glu	Phe	Asp	Glu	Ile	Val	Asp	Lys	Glu	Ile	Trp
305					310					315					320
Asn	Asp	Leu	Gly	Gly	Asp	Asp	Asp	Leu	Val	Leu	Arg	Arg	Ser	Gln	Val
			325					330						335	
Lys	Ala	Phe	Leu	Arg	Leu	Ala	Leu	Arg	Cys	Val	Arg	Tyr	Lys	Lys	Glu
			340					345					350		
Asp	Pro	Val	Ser	Gly	Met	Leu	Glu	Val	Ala	Lys	Glu	Leu	Lys	Leu	Ile
		355					360					365			
Glu	Lys	Leu	Ser												
		370													

<210> SEQ ID NO 29  
 <211> LENGTH: 357  
 <212> TYPE: PRT  
 <213> ORGANISM: Arabidopsis thaliana  
 <220> FEATURE:  
 <223> OTHER INFORMATION: putative kinase-like protein, kinase with  
 gatekeeper Cys

<400> SEQUENCE: 29

Met	Ser	Cys	Trp	Arg	Lys	Lys	Ser	Lys	Lys	Asn	Ser	Glu	Ala	Asn
1					5				10				15	
Gln	Arg	Gln	Arg	Trp	Phe	Gln	Glu	Asn	Gly	Lys	Val	Leu	Leu	Glu
		20					25					30		
Leu	Ile	Glu	Leu	Cys	Asn	Gly	Lys	Ser	Asn	Pro	Ile	Lys	Thr	Phe
		35				40					45			
Ala	Glu	Glu	Ile	Leu	Gln	Ala	Thr	Asp	Asn	Phe	Ser	Glu	Ser	Asn
	50				55					60				
Val	Ile	Arg	Phe	Asn	Phe	Met	Tyr	Arg	Gly	Ile	Leu	Gln	Asn	Arg
65				70					75				80	
Val	Leu	Ile	Lys	Arg	Ala	Thr	Trp	Asn	Tyr	Tyr	Lys	Ser	Asp	Thr
			85					90					95	
Glu	Lys	Ile	Cys	Arg	Asp	Ile	Ala	Val	Ser	Ser	Met	Val	Ser	Gly
		100					105					110		
Lys	Asn	Phe	Leu	Lys	Leu	Leu	Gly	Cys	Cys	Leu	Glu	Phe	Glu	His
	115					120					125			
Val	Leu	Val	Cys	Glu	Tyr	Ala	Glu	Arg	Ile	Pro	Phe	Asn	Thr	Pro
	130					135					140			
Pro	Glu	Met	Leu	Leu	Pro	Trp	Arg	Met	Arg	Ile	Lys	Ile	Ala	Lys
145			150						155				160	
Ile	Ala	Ile	Ala	Val	Ser	Tyr	Leu	His	Thr	Ala	Leu	Ser	Arg	Thr
			165				170						175	
Ile	His	Thr	Asp	Ile	Gln	Pro	Phe	Asn	Ile	Phe	Val	Asp	Ser	Asn
	180						185					190		
Thr	Ala	Lys	Leu	Ser	Asp	Phe	Cys	Leu	Cys	Ile	Ala	Ile	Pro	Glu
	195						200				205			
Glu	Thr	Phe	Val	Lys	Val	His	Ala	Asp	Arg	Val	Glu	Gly	Thr	Leu
	210					215					220			
Tyr	Leu	Glu	Tyr	Asn	Tyr	Ala	Ala	Thr	Gly	Leu	Ile	Thr	Glu	Tyr
225				230					235				240	
Asp	Val	Phe	Ser	Phe	Gly	Val	Leu	Leu	Gln	Asn	Phe	Phe	Thr	Arg
			245						250				255	
Tyr	Gly	Val	Val	Asp	Cys	Cys	Cys	Ser	Glu	Asp	Glu	Ser	Leu	Phe
		260					265					270		
Glu	Phe	Glu	Asp	Lys	Gln	Asn	Val	Met	Asn	Leu	Arg	Ile	Ser	Asp
	275						280					285		

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Ile Ser Lys Phe Val Glu Glu Gly Arg Ile Phe Asp Met Leu Asp Pro
290                295                300

Lys Met Leu Glu Ser Met Gly Asp Asp Glu Thr Glu Glu His Lys Ile
305                310                315                320

Arg Arg Met Lys Ala Val Leu Met Leu Ser Leu Arg Cys Thr Gly His
                325                330                335

Arg Gly Asp Val Pro Lys Met Met Glu Val Ala Lys Glu Leu Lys Arg
                340                345                350

Ile Glu Arg Trp Thr
355

<210> SEQ ID NO 30
<211> LENGTH: 340
<212> TYPE: PRT
<213> ORGANISM: Arabidopsis thaliana
<220> FEATURE:
<223> OTHER INFORMATION: protein kinase-like protein, kinase with
gatekeeper Cys

<400> SEQUENCE: 30

Met Leu Arg Leu Phe Arg Lys Lys Lys Lys Gln Lys Lys Glu Glu Glu
1      5      10      15

Ile Asn Leu Gln Lys Asn Gly Ser Leu Leu Leu Glu Glu Leu Ile Ala
20      25      30

Thr Ser Gly Gly Ile Tyr Asn Pro Ile Arg Thr Phe Ser Ser Asp Gln
35      40      45

Ile Leu Gln Ala Thr Asn His Phe Asp Trp Asn Tyr Val Ile Ser Glu
50      55      60

Asp Arg Phe Val Trp Tyr Lys Gly Met Ile Glu Asn Arg Pro Val Leu
65      70      75      80

Ile Lys Lys Phe Gln Asp Cys Ser Val Phe Asp Ala Asp Asn Phe Tyr
85      90      95

Arg Asp Ile Ala Val Ser Ser Leu Met Ser Ser His Lys Asn Val Leu
100     105     110

Lys Leu Leu Gly Cys Cys Leu Glu Phe Pro Arg Pro Val Leu Val Cys
115     120     125

Glu Tyr Pro Glu His Gly Ala Leu Asn Cys Ile Arg Cys Gly Lys Glu
130     135     140

Gly Val Arg Ser Phe Pro Trp Asn Val Arg Leu Arg Ile Ala Lys Glu
145     150     155     160

Ile Ala Asp Ala Val Ala Tyr Leu His Thr Glu Phe Pro Arg Thr Ile
165     170     175

Ile His Arg Asp Leu Lys Leu Ala Asn Ile Phe Leu Asp Glu Asn Trp
180     185     190

Ser Ala Lys Leu Ser Ser Phe Ser Leu Ser Ile Val Leu Pro Glu Gly
195     200     205

Glu Thr Gly Val Asn Asp Met Val Cys Arg Thr Ser Ser Tyr Ile Glu
210     215     220

Pro Asp Tyr Phe Asn Thr Gly Leu Val Thr Glu Asn Val Asp Ile Tyr
225     230     235     240

Ser Leu Gly Ile Ile Met Leu Ile Ile Leu Thr Gly Lys Ser Glu Tyr
245     250     255

Asn Ser Glu Val Ala Val Tyr Leu Pro Val Leu Pro Val Tyr Val Gly
260     265     270

Lys Phe Leu Glu Arg Gly Leu Leu Thr Glu Leu Ile Asp Pro Ser Ile

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275	280	285
Leu Asp Ser Thr Ser Asp Asp Ile Pro Lys His Ser Arg Leu Gln Met		
290	295	300
Glu Ala Phe Ile Glu Leu Ala Phe Arg Cys Val Arg Phe Arg Pro Gly		
305	310	315
Glu Asn Val Pro Arg Met Ile Asp Val Ala Lys Glu Leu Lys Lys Ile		
	325	330
		335
Glu Lys His Ile		
340		
<210> SEQ ID NO 31		
<211> LENGTH: 351		
<212> TYPE: PRT		
<213> ORGANISM: Arabidopsis thaliana		
<220> FEATURE:		
<223> OTHER INFORMATION: putative kinase-like protein, kinase with gatekeeper Cys		
<400> SEQUENCE: 31		
Met Lys Gly Phe Phe Lys Thr Glu Ser Glu Thr Arg Lys His Ser Asp		
1	5	10
Lys Asn Gly Ser Leu Leu His Glu Glu Leu Ile Ala Cys Ser Asp Gly		
	20	25
Lys Tyr Asn Pro Ile Arg Met Phe Ser Ser Asp Gln Ile Leu Lys Ala		
	35	40
Thr Asn Asn Phe Asp Ala Asp His Ile Ile Ala Lys Asp Arg Phe Ile		
	50	55
Trp Tyr Lys Gly Thr Ile Glu Glu Arg Arg Val Leu Ile Lys Lys Trp		
65	70	75
Glu Gly Asp Tyr Val Leu Phe Ser Ser Pro Glu Asn Val Tyr Arg Asp		
	85	90
Ile Ala Val Leu Ser Met Met Ser Ser His Lys Asn Val Leu Lys Leu		
	100	105
Leu Gly Cys Cys Val Glu Phe Tyr Lys Pro Val Leu Val Cys Glu Leu		
	115	120
Ala Glu Lys Gly Pro Leu Lys Leu Glu Asp Met Asp Gly Thr Pro Leu		
	130	135
Pro Trp Ser Ala Arg Leu Lys Ile Gly Lys Asp Ile Ala Asn Ala Val		
145	150	155
Ala Tyr Leu His Thr Ala Phe Pro Arg Val Ile Ile Asn Arg Asp Val		
	165	170
Arg Pro Gln Asn Ile Phe Leu Asp Glu Asp Gly Thr Ala Lys Leu Ser		
	180	185
Ser Phe Cys Leu Arg Ile Ser Ile Pro Glu Gly Glu Ser Ser Val Tyr		
	195	200
Asp Asp Lys Val Val Tyr Gly Val Ser Val Asp Pro Glu Tyr Asn Gly		
210	215	220
Thr Gly Leu Val Ser Glu Lys Phe Asp Val Tyr Ser Phe Gly Val Thr		
225	230	235
Met Leu Phe Leu Leu Gly Gly Glu Leu Gly Leu Thr Trp Leu Ser Ala		
	245	250
Ile Ile Gly Glu Phe Gly Phe Pro Phe Pro Gly Cys Gly Glu Glu Leu		
	260	265
Ala Asp Gln Phe Met Tyr Val Ile Asp Ser Asn Ile Trp Asn Gly Glu		
	275	285

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Ser Glu Ala Ser Ala Val Gln Val Glu Thr Phe Phe Gly Leu Ala Leu  
 290 295 300

Arg Cys Ile Arg Phe Trp Pro Gly Gln Asp Val Leu Thr Met Ile Asp  
 305 310 315 320

Val Ala Lys Glu Leu Lys Gly Ile Glu Glu Leu Phe Lys Ala Ser Ser  
 325 330 335

Ser Glu Gln Asp Lys Glu Gln Ile Asp Gln Val Asn Tyr Ser Val  
 340 345 350

<210> SEQ ID NO 32  
 <211> LENGTH: 690  
 <212> TYPE: PRT  
 <213> ORGANISM: Arabidopsis thaliana  
 <220> FEATURE:  
 <223> OTHER INFORMATION: similar to eukaryotic protein kinase domains,  
 kinase with gatekeeper Cys

<400> SEQUENCE: 32

Met Leu Leu Met Gln Ser Leu Leu Phe Thr Ile His Leu Ser Phe Leu  
 1 5 10 15

Phe Ser Leu Leu Cys Gln Ser Asn Cys Ser Cys Lys His His Ile Val  
 20 25 30

Pro Phe Ser Lys Val Ser Gln Glu Asp Ala Pro His Ala Ser His Ala  
 35 40 45

Gly Ala Tyr Pro Gln Arg Ile Gln Arg His Gly Thr Thr Asn Ser Glu  
 50 55 60

Ala Ile Leu Lys Phe Lys Glu Ser Leu Val Val Gly Gln Glu Asn Ala  
 65 70 75 80

Leu Ala Ser Trp Asn Ala Lys Ser Pro Pro Cys Thr Trp Ser Gly Val  
 85 90 95

Leu Cys Asn Gly Gly Ser Val Trp Arg Leu Gln Met Glu Asn Leu Glu  
 100 105 110

Leu Ser Gly Ser Ile Asp Ile Glu Ala Leu Ser Gly Leu Thr Ser Leu  
 115 120 125

Arg Thr Leu Ser Phe Met Asn Asn Lys Phe Glu Gly Pro Phe Pro Asp  
 130 135 140

Phe Lys Lys Leu Ala Ala Leu Lys Ser Leu Tyr Leu Ser Asn Asn Gln  
 145 150 155 160

Phe Gly Gly Asp Ile Pro Gly Asp Ala Phe Glu Gly Met Gly Trp Leu  
 165 170 175

Lys Lys Val His Leu Ala Gln Asn Lys Phe Thr Gly Gln Ile Pro Ser  
 180 185 190

Ser Val Ala Lys Leu Pro Lys Leu Leu Glu Leu Arg Leu Asp Gly Asn  
 195 200 205

Gln Phe Thr Gly Glu Ile Pro Glu Phe Glu His Gln Leu His Leu Leu  
 210 215 220

Asn Leu Ser Asn Asn Ala Leu Thr Gly Pro Ile Pro Glu Ser Leu Ser  
 225 230 235 240

Met Thr Asp Pro Lys Val Phe Glu Gly Asn Lys Gly Leu Tyr Gly Lys  
 245 250 255

Pro Leu Glu Thr Glu Cys Asp Ser Pro Tyr Ile Glu His Pro Pro Gln  
 260 265 270

Ser Glu Ala Arg Pro Lys Ser Ser Ser Arg Gly Pro Leu Val Ile Thr  
 275 280 285

Ala Ile Val Ala Ala Leu Thr Ile Leu Ile Ile Leu Gly Val Ile Phe  
 290 295 300

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Leu Leu Asn Arg Ser Tyr Lys Asn Lys Lys Pro Arg Leu Ala Val Glu  
 305 310 315 320  
 Thr Gly Pro Ser Ser Leu Gln Lys Lys Thr Gly Ile Arg Glu Ala Asp  
 325 330 335  
 Gln Ser Arg Arg Asp Arg Lys Lys Ala Asp His Arg Lys Gly Ser Gly  
 340 345 350  
 Thr Thr Lys Arg Met Gly Ala Ala Ala Gly Val Glu Asn Thr Lys Leu  
 355 360 365  
 Ser Phe Leu Arg Glu Asp Arg Glu Lys Phe Asp Leu Gln Asp Leu Leu  
 370 375 380  
 Lys Ala Ser Ala Glu Ile Leu Gly Ser Gly Cys Phe Gly Ala Ser Tyr  
 385 390 395 400  
 Lys Ala Val Leu Ser Ser Gly Gln Met Met Val Val Lys Arg Phe Lys  
 405 410 415  
 Gln Met Asn Asn Ala Gly Arg Asp Glu Phe Gln Glu His Met Lys Arg  
 420 425 430  
 Leu Gly Arg Leu Met His His Asn Leu Leu Ser Ile Val Ala Tyr Tyr  
 435 440 445  
 Tyr Arg Lys Glu Glu Lys Leu Leu Val Cys Asp Phe Ala Glu Arg Gly  
 450 455 460  
 Ser Leu Ala Ile Asn Leu His Ser Asn Gln Ser Leu Gly Lys Pro Ser  
 465 470 475 480  
 Leu Asp Trp Pro Thr Arg Leu Lys Ile Val Lys Gly Val Ala Lys Gly  
 485 490 495  
 Leu Phe Tyr Leu His Gln Asp Leu Pro Ser Leu Met Ala Pro His Gly  
 500 505 510  
 His Leu Lys Ser Ser Asn Val Leu Leu Thr Lys Thr Phe Glu Pro Leu  
 515 520 525  
 Leu Thr Asp Tyr Gly Leu Ile Pro Leu Ile Asn Gln Glu Lys Ala Gln  
 530 535 540  
 Met His Met Ala Ala Tyr Arg Ser Pro Glu Tyr Leu Gln His Arg Arg  
 545 550 555 560  
 Ile Thr Lys Lys Thr Asp Val Trp Gly Leu Gly Ile Leu Ile Leu Glu  
 565 570 575  
 Ile Leu Thr Gly Lys Phe Pro Ala Asn Phe Ser Gln Ser Ser Glu Glu  
 580 585 590  
 Asp Leu Ala Ser Trp Val Asn Ser Gly Phe His Gly Val Trp Ala Pro  
 595 600 605  
 Ser Leu Phe Asp Lys Gly Met Gly Lys Thr Ser His Cys Glu Gly Gln  
 610 615 620  
 Ile Leu Lys Leu Leu Thr Ile Gly Leu Asn Cys Cys Glu Pro Asp Val  
 625 630 635 640  
 Glu Lys Arg Leu Asp Ile Gly Gln Ala Val Glu Lys Ile Glu Glu Leu  
 645 650 655  
 Lys Glu Arg Glu Gly Asp Asp Asp Asp Phe Tyr Ser Thr Tyr Val Ser  
 660 665 670  
 Glu Thr Asp Gly Arg Ser Ser Lys Gly Glu Ser Cys Glu Ser Ile Ser  
 675 680 685  
 Phe Ala  
 690

&lt;210&gt; SEQ ID NO 33

&lt;211&gt; LENGTH: 662

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<212> TYPE: PRT  
 <213> ORGANISM: Arabidopsis thaliana  
 <220> FEATURE:  
 <223> OTHER INFORMATION: leucine-rich repeat protein kinase-like  
 protein, leucine-rich repeat transmembrane protein kinase-like  
 protein, kinase with gatekeeper Cys

<400> SEQUENCE: 33

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Met Pro Pro Met Gln Ala Arg Thr Leu Ser Val Tyr Asn Val Met Val
1          5          10          15

Pro Leu Val Cys Leu Leu Leu Phe Phe Ser Thr Pro Thr His Gly Leu
20          25          30

Ser Asp Ser Glu Ala Ile Leu Lys Phe Lys Glu Ser Leu Val Val Gly
35          40          45

Gln Glu Asn Ala Leu Ala Ser Trp Asn Ala Lys Ser Pro Pro Cys Thr
50          55          60

Trp Ser Gly Val Leu Cys Asn Gly Gly Ser Val Trp Arg Leu Gln Met
65          70          75          80

Glu Asn Leu Glu Leu Ser Gly Ser Ile Asp Ile Glu Ala Leu Ser Gly
85          90          95

Leu Thr Ser Leu Arg Thr Leu Ser Phe Met Asn Asn Lys Phe Glu Gly
100         105         110

Pro Phe Pro Asp Phe Lys Lys Leu Ala Ala Leu Lys Ser Leu Tyr Leu
115         120         125

Ser Asn Asn Gln Phe Gly Gly Asp Ile Pro Gly Asp Ala Phe Glu Gly
130         135         140

Met Gly Trp Leu Lys Lys Val His Leu Ala Gln Asn Lys Phe Thr Gly
145         150         155         160

Gln Ile Pro Ser Ser Val Ala Lys Leu Pro Lys Leu Leu Glu Leu Arg
165         170         175

Leu Asp Gly Asn Gln Phe Thr Gly Glu Ile Pro Glu Phe Glu His Gln
180         185         190

Leu His Leu Leu Asn Leu Ser Asn Asn Ala Leu Thr Gly Pro Ile Pro
195         200         205

Glu Ser Leu Ser Met Thr Asp Pro Lys Val Phe Glu Gly Asn Lys Gly
210         215         220

Leu Tyr Gly Lys Pro Leu Glu Thr Glu Cys Asp Ser Pro Tyr Ile Glu
225         230         235         240

His Pro Pro Gln Ser Glu Ala Arg Pro Lys Ser Ser Ser Arg Gly Pro
245         250         255

Leu Val Ile Thr Ala Ile Val Ala Ala Leu Thr Ile Leu Ile Ile Leu
260         265         270

Gly Val Ile Phe Leu Leu Asn Arg Ser Tyr Lys Asn Lys Lys Pro Arg
275         280         285

Leu Ala Val Glu Thr Gly Pro Ser Ser Leu Gln Lys Lys Thr Gly Ile
290         295         300

Arg Glu Ala Asp Gln Ser Arg Arg Asp Arg Lys Lys Ala Asp His Arg
305         310         315         320

Lys Gly Ser Gly Thr Thr Lys Arg Met Gly Ala Ala Ala Gly Val Glu
325         330         335

Asn Thr Lys Leu Ser Phe Leu Arg Glu Asp Arg Glu Lys Phe Asp Leu
340         345         350

Gln Asp Leu Leu Lys Ala Ser Ala Glu Ile Leu Gly Ser Gly Cys Phe
355         360         365

Gly Ala Ser Tyr Lys Ala Val Leu Ser Ser Gly Gln Met Met Val Val

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370	375	380
Lys Arg Phe Lys Gln Met Asn Asn Ala Gly Arg Asp Glu Phe Gln Glu		
385	390	395 400
His Met Lys Arg Leu Gly Arg Leu Met His His Asn Leu Leu Ser Ile		
	405	410 415
Val Ala Tyr Tyr Tyr Arg Lys Glu Glu Lys Leu Leu Val Cys Asp Phe		
	420	425 430
Ala Glu Arg Gly Ser Leu Ala Ile Asn Leu His Ser Asn Gln Ser Leu		
	435	440 445
Gly Lys Pro Ser Leu Asp Trp Pro Thr Arg Leu Lys Ile Val Lys Gly		
	450	455 460
Val Ala Lys Gly Leu Phe Tyr Leu His Gln Asp Leu Pro Ser Leu Met		
	465	470 475 480
Ala Pro His Gly His Leu Lys Ser Ser Asn Val Leu Leu Thr Lys Thr		
	485	490 495
Phe Glu Pro Leu Leu Thr Asp Tyr Gly Leu Ile Pro Leu Ile Asn Gln		
	500	505 510
Glu Lys Ala Gln Met His Met Ala Ala Tyr Arg Ser Pro Glu Tyr Leu		
	515	520 525
Gln His Arg Arg Ile Thr Lys Lys Thr Asp Val Trp Gly Leu Gly Ile		
	530	535 540
Leu Ile Leu Glu Ile Leu Thr Gly Lys Phe Pro Ala Asn Phe Ser Gln		
	545	550 555 560
Ser Ser Glu Glu Asp Leu Ala Ser Trp Val Asn Ser Gly Phe His Gly		
	565	570 575
Val Trp Ala Pro Ser Leu Phe Asp Lys Gly Met Gly Lys Thr Ser His		
	580	585 590
Cys Glu Gly Gln Ile Leu Lys Leu Leu Thr Ile Gly Leu Asn Cys Cys		
	595	600 605
Glu Pro Asp Val Glu Lys Arg Leu Asp Ile Gly Gln Ala Val Glu Lys		
	610	615 620
Ile Glu Glu Leu Lys Glu Arg Glu Gly Asp Asp Asp Asp Phe Tyr Ser		
	625	630 635 640
Thr Tyr Val Ser Glu Thr Asp Gly Arg Ser Ser Lys Gly Glu Ser Cys		
	645	650 655
Glu Ser Ile Ser Phe Ala		
	660	

&lt;210&gt; SEQ ID NO 34

&lt;211&gt; LENGTH: 1266

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Arabidopsis thaliana

&lt;220&gt; FEATURE:

<223> OTHER INFORMATION: Ser-Thr protein kinase-like protein, kinase  
with gatekeeper Cys

&lt;400&gt; SEQUENCE: 34

Met Ile Phe His Gly Ser Lys Ala Val Lys Glu Ala Gln Ser Trp Gln
1 5 10 15
Glu Ala Gln Phe Glu Leu His Ser Glu Ser His Gly Ser Leu Ser Ile
20 25 30
Asp Asn Arg Ile Arg Val Arg Asp Val Ser Gln Asp Thr Thr Phe Ser
35 40 45
Gly Tyr Arg Cys Phe Ile Asp Gly Ser Trp Lys Ala Ser Asp Gln Phe
50 55 60

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Ser	Gly	Thr	Gly	Trp	Phe	Cys	Leu	Ser	Ser	Leu	Gly	Glu	Ser	Pro	Thr	65	70	75	80
Met	Gly	Ala	Val	Asn	Val	Arg	Arg	Ser	Leu	Ser	Pro	Leu	His	Thr	Glu	85	90	95	
Met	Glu	Ala	Leu	Leu	Trp	Ala	Met	Lys	Cys	Met	Ile	Gly	Ala	Asp	Asn	100	105	110	
Gln	Asn	Val	Ala	Phe	Phe	Thr	Asp	Cys	Ser	Cys	His	Arg	Phe	Gly	Glu	115	120	125	
Met	Glu	Asp	Ser	Ser	Ser	Ile	Asp	Ser	Ile	Leu	Glu	Phe	Leu	Arg	Lys	130	135	140	
Asn	His	Phe	Met	Arg	Ala	Glu	Ala	Ala	Leu	Ile	Ser	Glu	Leu	Ser	Lys	145	150	155	160
Lys	Pro	Ser	Ser	Asn	Gly	Ser	Leu	Gln	Lys	Leu	Asn	Phe	Glu	Asp	Asn	165	170	175	
Cys	Val	Ser	Lys	Leu	Leu	Asp	Lys	Lys	Lys	Gln	Gly	Gly	Ser	Ser	Gln	180	185	190	
Ala	Leu	Gly	Leu	His	Asn	Asp	Ser	His	Ile	Ser	Asp	Glu	Leu	Val	Val	195	200	205	
Lys	Glu	Ile	Gln	Cys	Gly	Ala	Ala	Asn	Asn	Leu	His	Glu	Ser	Asn	Leu	210	215	220	
Met	Asn	Asp	Val	Ser	Val	Gln	Thr	Gln	Ser	Gly	Asn	Ala	Asp	Phe	Trp	225	230	235	240
Glu	Glu	Arg	Phe	Thr	Phe	Ala	Glu	Gly	Phe	Glu	Asp	Thr	Glu	Leu	Asp	245	250	255	
Leu	Pro	Pro	Trp	Asn	His	Thr	Ser	Thr	Asp	Ile	Val	Ala	Asp	Ser	Glu	260	265	270	
Glu	Tyr	Ser	Ile	Asn	Pro	Ser	Lys	Arg	Gly	Phe	Val	Asn	Pro	Arg	Ser	275	280	285	
Ser	Lys	Gln	Ser	Ser	His	Glu	Lys	Val	Pro	Glu	Pro	Gly	Lys	Ser	Asn	290	295	300	
Lys	Val	Val	Val	Glu	Asp	Val	Phe	Ser	Ser	Phe	Glu	Lys	Ile	Arg	Thr	305	310	315	320
Gly	Ser	Ser	Ser	Gln	Val	Ser	Gln	Tyr	Asp	His	Gly	Lys	Ala	Cys	Gln	325	330	335	
Ser	Leu	Glu	Val	Asp	Asn	Lys	Val	Gly	Asn	Ser	Ala	Ile	Gln	Glu	Gly	340	345	350	
Phe	Val	Thr	Thr	Ser	Trp	Ser	Arg	Ser	Glu	Glu	Asn	Ile	Gly	Ala	Ser	355	360	365	
Pro	Asp	His	Trp	Lys	Asp	Cys	Ser	Val	Thr	Thr	Val	Phe	Pro	Leu	Ser	370	375	380	
Lys	Gly	Ser	Thr	Ser	Thr	Lys	Asp	Asn	Gly	Val	Ala	Ile	Leu	Asp	Lys	385	390	395	400
Trp	Gln	Gly	Lys	Lys	Leu	Val	Gly	Ala	Ser	Asp	Ser	Arg	Ile	Leu	Ile	405	410	415	
Lys	Glu	Gln	Glu	Asp	Asp	Val	Ala	Thr	Ala	Leu	Tyr	Leu	Gly	Lys	Ser	420	425	430	
Gln	Ser	Gly	Tyr	Glu	His	Lys	Ile	Pro	Ser	Ser	Leu	Ala	Phe	Ser	Leu	435	440	445	
Ala	His	Asp	Ala	Pro	Arg	Glu	Asp	Leu	Pro	Arg	Leu	Pro	His	Val	Lys	450	455	460	
Ile	Lys	Ser	Glu	Asp	Lys	Leu	Met	Asn	Phe	Thr	Trp	Glu	Glu	Lys	His	465	470	475	480
Glu	Arg	Asp	Ile	Leu	Asp	Glu	Lys	Leu	Ile	Asn	Thr	Asp	Asn	Ala	Phe				

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485								490					495				
Leu	Leu	Gly	Ser	Tyr	Leu	Asp	Val	Pro	Ile	Gly	Gln	Glu	Ile	Asn	Ser		
			500					505					510				
Ser	Gly	Gly	Lys	Met	Ala	Gly	Gly	Gly	Asn	Trp	Leu	Ser	Val	Ser	His		
		515					520					525					
Gly	Ile	Ala	Asp	Asp	Ala	Ser	Asp	Leu	Ile	Phe	Gly	Phe	Gly	Asp	Gly		
	530					535					540						
Leu	Gly	Ala	Leu	Asn	Glu	His	Ser	Asn	Glu	Tyr	Trp	Asp	Ser	Asp	Glu		
545					550					555					560		
Tyr	Asp	Asp	Asp	Asp	Asp	Val	Gly	Tyr	Ile	Arg	Gln	Pro	Ile	Glu	Asp		
				565					570					575			
Glu	Ala	Trp	Phe	Leu	Gly	His	Glu	Val	Asp	Tyr	Pro	Ser	Asp	Asn	Glu		
			580					585					590				
Lys	Gly	Thr	Glu	His	Gly	Ser	Val	Pro	Asp	Thr	Gln	Asp	Lys	Ser	Gln		
		595					600					605					
Thr	Lys	Asn	Asp	Asp	Asp	His	Ser	Phe	Ala	Glu	Glu	Asp	Ser	Tyr	Phe		
	610					615					620						
Ser	Gly	Glu	Gln	Tyr	Val	Leu	Ala	Lys	Gly	Ile	Glu	Pro	Val	Thr	Ala		
625					630					635					640		
Ser	Asn	Asp	Pro	Met	Gly	Leu	Ser	Met	Thr	Glu	Thr	Tyr	Ser	Thr	Thr		
				645					650					655			
Lys	Gln	Ala	Asp	Leu	Val	Ala	Arg	Tyr	Asp	Gly	Gln	Leu	Met	Asp	Ala		
			660					665					670				
Glu	Glu	Leu	Ser	Leu	Met	Asp	Thr	Glu	Pro	Val	Trp	Lys	Gly	Phe	Val		
		675					680					685					
Ser	His	Glu	Asn	Asp	Val	Ile	Leu	Leu	Lys	Lys	Gly	Lys	Val	Glu	Asp		
	690					695					700						
Asn	Ser	Gly	Arg	Ile	Cys	Arg	Lys	Asp	Ile	Arg	Ala	Glu	Asp	Asp	Arg		
705				710						715					720		
Asn	Ala	Ala	Val	Arg	Ser	Ile	Gly	Val	Gly	Met	Ser	Asp	Asp	Val	Asp		
				725					730					735			
Asp	Asn	Gly	Ser	Ile	Ile	Pro	Glu	Tyr	Phe	Pro	Gly	Glu	Gly	Ser	Glu		
			740					745					750				
Trp	Asp	Leu	Glu	Leu	Leu	Pro	Tyr	Arg	Gly	Val	Gly	Val	Ala	Gly	Val		
		755				760						765					
Lys	Pro	Pro	Pro	Gly	Lys	Gly	Ala	Ser	Met	Leu	Leu	Lys	Asn	Phe	Ala		
	770				775						780						
Asp	Gly	Gly	Phe	Ser	Phe	Pro	Ser	Pro	Val	Ala	Asp	Arg	Gln	Lys	Ser		
785				790						795					800		
Gln	Asp	Asp	Ser	Ala	Asn	Pro	Glu	Trp	Ser	Asn	His	Cys	Asp	Ala	Val		
				805					810					815			
Val	Arg	Asn	Glu	Ser	Asp	Glu	Pro	Lys	Gly	Leu	Ile	Gln	Ser	Asp	Ser		
			820					825					830				
Met	Ile	Val	Ser	Ser	Thr	Lys	Arg	Cys	Ser	Gly	Ser	Ser	Ser	Glu	Arg		
		835					840					845					
Asn	Leu	Arg	Asp	Met	Asp	Asp	Glu	Lys	Val	Ala	Ser	Ser	Arg	Asn	Ser		
	850					855					860						
Ser	Pro	Ser	Ala	Leu	Ser	His	Ser	Ser	Asp	Thr	Gly	Arg	Glu	His	Lys		
865					870					875					880		
Glu	Glu	Asp	Glu	Glu	Glu	Thr	Ser	His	Gly	Pro	Glu	Glu	Asp	Pro	Gly		
			885						890					895			
Thr	Ser	Phe	Glu	Asp	Glu	Asp	Ala	Ile	Val	Val	Gln	Glu	Gln	Val	Arg		
			900					905					910				

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Gln Ile Gln Ala Gln Glu Gln Asp Phe Glu Thr Phe Asn Leu Lys Ile  
 915 920 925  
 Val His Arg Lys Asn Arg Thr Gly Phe Glu Glu Asp Lys Asn Phe His  
 930 935 940  
 Val Val Leu Asn Ser Val Ile Ala Gly Arg Tyr His Val Thr Glu His  
 945 950 955 960  
 Leu Gly Ser Ala Ala Phe Ser Lys Ala Ile Gln Ala His Asp Leu His  
 965 970 975  
 Thr Gly Ile Asp Val Cys Val Lys Ile Ile Lys Asn Asn Lys Asp Phe  
 980 985 990  
 Phe Asp Gln Ser Leu Asp Glu Ile Lys Leu Leu Lys Tyr Val Asn Gln  
 995 1000 1005  
 His Asp Pro Ala Asp Lys Tyr His Leu Leu Arg Leu Tyr Asp Tyr  
 1010 1015 1020  
 Phe Tyr Phe Arg Glu His Leu Leu Ile Val Cys Glu Leu Leu Lys  
 1025 1030 1035  
 Ala Asn Leu Tyr Glu Phe Gln Lys Phe Asn Arg Glu Ser Gly Gly  
 1040 1045 1050  
 Glu Val Tyr Phe Thr Met Pro Arg Leu Gln Ser Ile Thr Ile Gln  
 1055 1060 1065  
 Cys Leu Glu Ala Leu Asn Phe Leu His Gly Leu Gly Leu Ile His  
 1070 1075 1080  
 Cys Asp Leu Lys Pro Glu Asn Ile Leu Ile Lys Ser Tyr Ser Arg  
 1085 1090 1095  
 Cys Glu Ile Lys Val Ile Asp Leu Gly Ser Ser Cys Phe Glu Thr  
 1100 1105 1110  
 Asp His Leu Cys Ser Tyr Val Gln Ser Arg Ser Tyr Arg Ala Pro  
 1115 1120 1125  
 Glu Val Ile Leu Gly Leu Pro Tyr Asp Lys Lys Ile Asp Ile Trp  
 1130 1135 1140  
 Ser Leu Gly Cys Ile Leu Ala Glu Leu Cys Thr Gly Asn Val Leu  
 1145 1150 1155  
 Phe Gln Asn Asp Ser Pro Ala Thr Leu Leu Ala Arg Val Ile Gly  
 1160 1165 1170  
 Ile Ile Gly Ser Ile Asp Gln Glu Met Leu Ala Lys Gly Arg Asp  
 1175 1180 1185  
 Thr Cys Lys Tyr Phe Thr Lys Asn His Leu Leu Tyr Glu Arg Asn  
 1190 1195 1200  
 Gln Glu Ser Asn Asn Leu Glu Tyr Leu Ile Pro Lys Lys Ser Ser  
 1205 1210 1215  
 Leu Arg Arg Arg Leu Pro Met Gly Asp Gln Gly Phe Ile Asp Phe  
 1220 1225 1230  
 Val Ala Tyr Leu Leu Gln Val Asp Pro Lys Lys Arg Pro Ser Ala  
 1235 1240 1245  
 Phe Glu Ala Leu Lys His Pro Trp Leu Thr Tyr Pro Tyr Glu Pro  
 1250 1255 1260  
 Ile Ser Ala  
 1265

&lt;210&gt; SEQ ID NO 35

&lt;211&gt; LENGTH: 1157

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Arabidopsis thaliana

&lt;220&gt; FEATURE:

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<223> OTHER INFORMATION: hypothetical kinase-like protein, kinase with gatekeeper Cys

<400> SEQUENCE: 35

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Met Thr Asp Gln Ser Ser Val Asp Gly Ile Leu Glu Phe Leu Arg Asn
1          5          10          15

Asn Arg Phe Ser Gln Ala Glu Glu Ala Leu Arg Asn Glu Leu Asn Asn
          20          25          30

Arg Ser Asp Ile Asn Gly Phe Leu Gln Lys Leu Lys Leu Glu Asp Lys
          35          40          45

Asp Ser Asn Glu Lys Ala Ala Gly Asn Glu Leu Arg Arg Ser Gly Ser
          50          55          60

Arg Asp Ser Glu Val Ser Lys Glu Leu Ile Val Lys Glu Val Asp Cys
          65          70          75          80

Gly Thr Ser Thr Asn Gly Ser Val Ile Lys Trp Glu Asn Gly Ala Thr
          85          90          95

Ala Asp Asn Pro Ser Lys Lys Glu Pro Val Val Ser Ser Glu Met Ser
          100         105         110

Phe Thr Phe Ser Lys Asn Ser Gly Asp Ala Ala Ala Pro Pro Asp Ala
          115         120         125

His Ser Tyr Lys Phe Thr Ser Arg Asn Gly Thr Val Glu Pro Ser Arg
          130         135         140

Asn Ile Asp Asp Ser Ser Ser Ser Ser Leu Val Asp Leu Tyr Ala Phe
          145         150         155         160

Glu Gln Ser Arg His Gly Asn Phe Ala Asp Ile Asp Lys Lys Ile Val
          165         170         175

Glu Thr Gly Glu Asp Ile Val Phe Phe Gly Asn Lys Ser Thr Ser Trp
          180         185         190

Ser Gly Asn Ser Ser Lys Gly Asn Ser Gly Ser Lys Ile Lys Glu Pro
          195         200         205

Asn Glu Ile His Arg Leu Val Glu Asn Ser Gly Lys His Asp Ser Tyr
          210         215         220

Lys Gly Ser Ile Leu Leu Arg Ser Glu Asp Val Val Asp Thr Ser Ala
          225         230         235         240

Asn Trp Arg Glu Cys Ser Val Lys Thr Leu Phe Gln Ser Ser Arg Gly
          245         250         255

Asp Ala Ser Asn Ser Tyr Asn Leu Val Ser Ser Ser Asp Lys Arg Glu
          260         265         270

Gly Lys Lys Lys Ala Asp Ile Ser Asp Val Arg Val Ala Ile Lys Glu
          275         280         285

Gln Glu Ser Glu Val Ala Arg Ala Leu Phe Phe Gly Lys Ser Gln Ser
          290         295         300

Thr Phe Asp Asp Lys Asn Ile Ser Ser Leu Gly Phe Pro Leu Val Tyr
          305         310         315         320

Asp Thr Arg Lys Glu Glu Phe Pro Arg Leu Pro Pro Val Lys Leu Lys
          325         330         335

Ser Glu Asp Asn Pro Leu Ser Leu His Cys Glu Glu Lys Phe Glu Arg
          340         345         350

Asp Gly Ser Gly Pro Arg Leu Ile Asn Asp Glu Asp Ala Leu Leu Ile
          355         360         365

Gly Ser Tyr Leu Asp Val Pro Ile Gly Gln Glu Ile Ser Ser Ser Val
          370         375         380

Ser Gln Gly Ile Ala Glu Asp Ala Ser Asp Leu Val Ser Gly Phe Ala
          385         390         395         400

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Thr Ile Gly Asp Gly Leu Ser Glu Ser Val Asp Tyr Arg Asn Glu Tyr  
 405 410 415  
 Trp Asp Ser Asp Glu Tyr Glu Asp Asp Asp Ile Gly Tyr Val Arg  
 420 425 430  
 Gln Pro Ile Glu Asp Glu Pro Trp Phe Leu Ala His Glu Ile Asp Tyr  
 435 440 445  
 Pro Ser Asp His Glu Lys Gly Thr Thr Arg Gly Ser Pro Asp His His  
 450 455 460  
 Glu Arg Asp Ala Asn Lys Asp Ala Asp Asp Gln Ser Tyr Ala Glu Glu  
 465 470 475 480  
 Ala Ser Tyr Ile Ser Gly Glu Gln Tyr Leu Gln Ser Lys Asp Ala Glu  
 485 490 495  
 Pro Ile Ser Ser Glu Asn Asp Arg Arg Leu Thr Val Ser Glu Ile Tyr  
 500 505 510  
 Pro Ala Ser Lys Lys Asn Asp Leu Leu Ala Gln Tyr Asp Gly His Leu  
 515 520 525  
 Met Asp Glu Glu Leu Leu Ser Ser Met Arg Asp Glu Pro Val Trp Gln  
 530 535 540  
 Gly Phe Val Ala Gln Ser Asn Glu Leu Leu Met Leu Gly Asp Lys Lys  
 545 550 555 560  
 Gly Ile Asn Val His Arg Lys Ser His Arg Asp Asp Val Tyr Val Glu  
 565 570 575  
 Asp Asp Gln His Asp Ser Val Arg Ser Ile Gly Val Gly Ile Asn Ser  
 580 585 590  
 Asp Ala Ala Asp Phe Gly Ser Glu Val Arg Asp Ser Leu Ala Gly Gly  
 595 600 605  
 Ser Ser Glu Gly Asp Phe Glu Tyr Ser Arg Asp His Asp Pro Val Ala  
 610 615 620  
 Ser Arg Phe Lys Gln Leu Tyr Ser Glu Ser Asp Lys Lys His Ile Asp  
 625 630 635 640  
 Ala Pro Asn Lys Asn Lys Gln Gln Ala Ser Lys Asn Asp Gly Pro Asp  
 645 650 655  
 Tyr Ile Ala Asp Asn Asp Ser Ser Gly Ser Phe His Val Lys Ile Gln  
 660 665 670  
 Thr Asp Gly Gly Phe Ser Phe Gly Ser Ser Gln Lys Asp Gly Gln Ser  
 675 680 685  
 Met His Ala Glu Ser Ser Lys Ser Leu Trp Ser Gly Asn His Glu Thr  
 690 695 700  
 Val Thr Arg Asp Arg Asn Thr Glu Arg Leu Ser Ala Ser Thr Ala Met  
 705 710 715 720  
 Asp Asp Met Val Ala Thr Trp Arg Arg Lys Ser Ser Asp Ser Ser Ser  
 725 730 735  
 Ser His Ser Ser Val Lys Asp Asn Asn Ala Thr Ser Ile Lys Ser Leu  
 740 745 750  
 Asn Ser Ser Pro Ser Ser Leu Ser Asn Tyr Ala Cys Glu Glu Arg Lys  
 755 760 765  
 His Ala Asp Lys Glu Asp Asp Arg Asn Asp Ser Ser Glu Ile Glu Asp  
 770 775 780  
 Asp Asn Ala Thr Ala Leu Asp Asp Glu Glu Ala Val Ala Val Gln Glu  
 785 790 795 800  
 Gln Val Arg Gln Ile Lys Ala Gln Glu Glu Glu Phe Glu Thr Phe Asp  
 805 810 815

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Leu Lys Ile Val His Arg Lys Asn Arg Thr Gly Phe Glu Glu Glu Lys
      820                        825                        830

Asn Phe Asn Val Val Leu Asn Ser Val Ile Ala Gly Arg Tyr His Val
      835                        840                        845

Thr Glu Tyr Leu Gly Ser Ala Ala Phe Ser Lys Ala Ile Gln Ala His
      850                        855                        860

Asp Leu Gln Thr Gly Met Asp Val Cys Ile Lys Ile Ile Lys Asn Asn
      865                        870                        875                        880

Lys Asp Phe Phe Asp Gln Ser Leu Asp Glu Ile Lys Leu Leu Lys Tyr
      885                        890                        895

Val Asn Lys His Asp Pro Ala Asp Lys Tyr His Leu Leu Arg Leu Tyr
      900                        905                        910

Asp Tyr Phe Tyr Tyr Arg Glu His Leu Leu Ile Val Cys Glu Leu Leu
      915                        920                        925

Lys Ala Asn Leu Tyr Glu Phe His Lys Phe Asn Arg Glu Ser Gly Gly
      930                        935                        940

Glu Val Tyr Phe Thr Met Pro Arg Leu Gln Ser Ile Thr Ile Gln Cys
      945                        950                        955                        960

Leu Glu Ser Leu Gln Phe Leu His Gly Leu Gly Leu Ile His Cys Asp
      965                        970                        975

Leu Lys Pro Glu Asn Ile Leu Val Lys Ser Tyr Ser Arg Cys Glu Ile
      980                        985                        990

Lys Val Ile Asp Leu Gly Ser Ser Cys Phe Glu Thr Asp His Leu Cys
      995                        1000                        1005

Ser Tyr Val Gln Ser Arg Ser Tyr Arg Ala Pro Glu Val Ile Leu
      1010                        1015                        1020

Gly Leu Pro Tyr Asp Lys Lys Ile Asp Val Trp Ser Leu Gly Cys
      1025                        1030                        1035

Ile Leu Ala Glu Leu Cys Thr Gly Asn Val Leu Phe Gln Asn Asp
      1040                        1045                        1050

Ser Pro Ala Ser Leu Leu Ala Arg Val Met Gly Ile Val Gly Ser
      1055                        1060                        1065

Phe Asp Asn Glu Met Leu Thr Lys Gly Arg Asp Ser His Lys Tyr
      1070                        1075                        1080

Phe Thr Lys Asn Arg Met Leu Tyr Glu Arg Asn Gln Glu Ser Asn
      1085                        1090                        1095

Arg Leu Glu Tyr Leu Ile Pro Lys Arg Thr Ser Leu Arg His Arg
      1100                        1105                        1110

Leu Pro Met Gly Asp Gln Gly Phe Thr Asp Phe Val Ala His Leu
      1115                        1120                        1125

Leu Glu Ile Asn Pro Lys Lys Arg Pro Ser Ala Ala Glu Ala Leu
      1130                        1135                        1140

Lys His Pro Trp Leu Ser Tyr Pro Tyr Glu Pro Ile Ser Ala
      1145                        1150                        1155

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&lt;210&gt; SEQ ID NO 36

&lt;211&gt; LENGTH: 1155

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Arabidopsis thaliana

&lt;220&gt; FEATURE:

<223> OTHER INFORMATION: putative protein kinase-like protein, kinase  
with gatekeeper Cys

&lt;400&gt; SEQUENCE: 36

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Met Ala Asp Gln Ser Ser Val Asp Gly Ile Leu Glu Phe Leu Arg Asn
1           5           10           15

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Asn	Arg	Phe	Ser	Asn	Ala	Glu	Glu	Ala	Leu	Arg	Asn	Glu	Leu	Ser	Asn
			20					25					30		
Arg	Ser	Asp	Ile	Asn	Gly	Phe	Leu	Gln	Lys	Leu	Met	Leu	Glu	Glu	Lys
		35					40					45			
Asp	Ser	Ser	Lys	Asp	Ser	Asn	Glu	Arg	Ala	Asn	Gly	Lys	Glu	Leu	Arg
		50				55				60					
Arg	Ser	Gly	Ser	Arg	Asp	Ser	Glu	Val	Ser	Lys	Glu	Leu	Val	Val	Lys
				70					75						80
Glu	Val	Asp	Cys	Gly	Thr	Ser	Thr	Thr	Gly	Ser	Val	Ile	Lys	Trp	Glu
			85						90					95	
Asn	Gly	Ala	Ala	Ala	Glu	Asn	Pro	Ser	Lys	Lys	Glu	Thr	Phe	Val	Pro
			100					105					110		
Ser	Glu	Met	Ser	Phe	Thr	Phe	Ser	Lys	Asn	Ser	Gly	Asp	Ala	Ala	Ala
		115					120					125			
Pro	Pro	Asp	Ala	His	Ser	Tyr	Glu	Phe	Thr	Ser	Gly	Asn	Gly	Thr	Leu
		130				135					140				
Glu	Pro	Tyr	Gly	Asn	Ile	Asp	Asp	Asn	Ser	Ser	Ser	Ser	Leu	Val	Asp
				150					155						160
Ser	Tyr	Ala	Ile	Glu	Gln	Leu	Ala	Asp	Ile	Asp	Lys	Lys	Ile	Val	Glu
			165						170					175	
Thr	Gly	Glu	Asp	Ile	Val	Phe	Phe	Gly	Asn	Lys	Ser	Thr	Leu	Leu	Ser
			180					185					190		
Gly	Asn	Ser	Ser	Lys	Gly	Asn	Ser	Gly	Ser	Lys	Ile	Lys	Lys	Pro	Asn
			195				200					205			
Glu	Ile	Asp	Gln	Leu	Gly	Glu	Ile	Phe	Gly	Lys	His	Asp	Ser	Tyr	Lys
					215						220				
Gly	Ser	Val	Leu	Leu	Arg	Thr	Glu	Asp	Val	Ile	Asp	Thr	Ser	Glu	Asn
					230				235						240
Trp	Lys	Glu	Arg	Ser	Val	Lys	Thr	Leu	Phe	Gln	Ser	Ser	Arg	Gly	Asp
			245						250					255	
Ala	Ser	Asn	Ser	Tyr	Asn	Leu	Val	Ser	Ser	Ser	Asp	Lys	Arg	Glu	Gly
			260					265				270			
Lys	Lys	Lys	Ala	Glu	Ile	Ser	Asp	Val	Arg	Val	Ala	Ile	Lys	Glu	Gln
			275				280					285			
Glu	Ser	Glu	Val	Ala	Arg	Ala	Leu	Phe	Phe	Gly	Lys	Ser	Gln	Ser	Thr
					295					300					
Phe	Asp	Asp	Lys	Asn	Ile	Ser	Ser	Leu	Gly	Phe	Pro	Leu	Val	Phe	Asp
				310						315					320
Thr	Arg	Lys	Glu	Glu	Phe	Pro	Arg	Leu	Pro	Pro	Val	Lys	Leu	Lys	Ser
			325					330					335		
Glu	Asp	Asn	Pro	Leu	Ser	Leu	His	Cys	Glu	Glu	Lys	Phe	Glu	Arg	Asp
			340					345				350			
Gly	Ser	Gly	Pro	Arg	Leu	Ile	Asn	Asp	Asp	Glu	Ala	Leu	Leu	Ile	Gly
		355					360					365			
Ser	Tyr	Leu	Asp	Val	Pro	Ile	Gly	Gln	G						

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Glu	Thr	Trp	Phe	Leu	Ala	His	Glu	Ile	Asp	Tyr	Pro	Ser	Asp	His	Glu
		435					440					445			
Lys	Gly	Thr	Thr	Arg	Gly	Ser	Pro	Asp	His	His	Asp	Arg	Asp	Ala	Asn
	450					455					460				
Lys	Asp	Glu	Asp	Asp	Gln	Ser	Tyr	Ala	Glu	Asp	Glu	Ser	Tyr	Leu	Ser
	465				470					475					480
Gly	Glu	Arg	Tyr	Leu	Gln	Ser	Lys	Asp	Ala	Glu	Pro	Ile	Ser	Ser	Glu
				485					490					495	
Asn	Asp	Arg	Arg	Leu	Thr	Val	Ser	Glu	Ile	Tyr	Pro	Ala	Cys	Lys	Lys
			500					505					510		
Asn	Asp	Leu	Leu	Ala	Gln	Tyr	Asp	Gly	Gln	Leu	Met	Asp	Glu	Asp	Leu
		515					520					525			
Leu	Asn	Ser	Met	Arg	Thr	Glu	Pro	Val	Trp	Gln	Gly	Phe	Val	Ala	Gln
	530					535					540				
Ser	Asn	Glu	Leu	Val	Met	Leu	Gly	Asp	Lys	Lys	Gly	Ile	Asn	Val	His
	545				550					555					560
Arg	Lys	Ser	His	Leu	Asp	Asp	Val	Tyr	Val	Glu	Asp	Asp	Gln	His	Asp
				565					570					575	
Ser	Val	Arg	Ser	Ile	Gly	Val	Gly	Ile	Asn	Ser	Asp	Ala	Ala	Asp	Phe
			580					585					590		
Gly	Ser	Glu	Val	Arg	Asp	Ser	Leu	Ala	Gly	Gly	Ser	Ser	Glu	Gly	Asp
		595					600					605			
Phe	Glu	Tyr	Ser	Arg	Asp	His	Asp	Pro	Val	Ala	Ser	Arg	Phe	Lys	Gln
	610					615					620				
Leu	Tyr	Ser	Glu	Ser	Asp	Lys	Lys	His	Ile	Asp	Gly	Gln	Asn	Lys	Asn
	625				630					635					640
Lys	Gln	Lys	Ala	Ser	Lys	Asn	Asp	Ser	Gly	Gly	Ser	Phe	His	Val	Lys
				645					650					655	
Ile	Gln	Thr	Asp	Gly	Asp	Phe	Ser	Phe	Gly	Ser	Ser	Gln	Lys	Asp	Gly
			660					665					670		
Gln	Leu	Met	His	Ala	Glu	Ser	Ser	Lys	Ser	Leu	Trp	Ser	Gly	Asn	Arg
		675					680					685			
Glu	Thr	Val	Thr	Arg	Asp	Arg	Asn	Thr	Glu	Leu	Leu	Ser	Ala	Ser	Thr
	690					695					700				
Ala	Thr	Asp	Asp	Met	Val	Ala	Thr	Trp	Arg	Gln	Lys	Ser	Ser	Asp	Ser
	705				710					715					720
Ser	Ser	Ser	Arg	Ser	Ser	Val	Lys	Glu	Asn	Asn	Ala	Thr	Ser	Ile	Lys
				725					730					735	
Ser	Val	Asn	Ser	Ser	Pro	Ser	Ser	Leu	Ser	Asn	Tyr	Ala	Arg	Gly	Glu
		740						745					750		
Arg	Lys	His	Ala	Glu	Lys	Glu	Asn	Asp	Ser	Ser	Glu	Arg	Glu	Asp	Gly
		755					760					765			
His	Ala	Thr	Ala	Leu	Asp	Asp	Glu	Glu	Ala	Val	Ala	Val	Gln	Glu	Gln
	770				775						780				
Val	Arg	Gln	Ile	Lys	Ala	Gln	Glu	Glu	Glu	Phe	Glu	Thr	Phe	Asp	Leu
	785				790					795					800
Lys	Ile	Val	His	Arg	Lys	Asn	Arg	Thr	Gly	Phe	Glu	Glu	Glu	Lys	Asn
				805					810					815	
Phe	Asn	Val	Val	Leu	Asn	Ser	Val	Ile	Ala	Gly	Arg	Tyr	His	Val	Thr
		820						825					830		
Glu	Tyr	Leu	Gly	Ser	Ala	Ala	Phe	Ser	Lys	Ala	Ile	Gln	Ala	His	Asp
	835						840					845			
Leu	Gln	Thr	Gly	Met	Asp	Val	Cys	Ile	Lys	Ile	Ile	Lys	Asn	Asn	Lys

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850	855	860
Asp Phe Phe Asp Gln Ser Leu Asp Glu Ile Lys Leu Leu Lys Tyr Val		
865	870	875 880
Asn Lys His Asp Pro Ala Asp Lys Tyr His Leu Leu Arg Leu Tyr Asp		
	885	890 895
Tyr Phe Tyr Tyr Arg Glu His Leu Leu Ile Val Cys Glu Leu Leu Lys		
	900	905 910
Ala Asn Leu Tyr Glu Phe His Lys Phe Asn Arg Glu Ser Gly Gly Glu		
	915	920 925
Val Tyr Phe Thr Met Pro Arg Leu Gln Ser Ile Thr Ile Gln Cys Leu		
	930	935 940
Glu Ser Leu Gln Phe Leu His Gly Leu Gly Leu Ile His Cys Asp Leu		
	945	950 955 960
Lys Pro Glu Asn Ile Leu Val Lys Ser Tyr Ser Arg Cys Glu Ile Lys		
	965	970 975
Val Ile Asp Leu Gly Ser Ser Cys Phe Glu Thr Asp His Leu Cys Ser		
	980	985 990
Tyr Val Gln Ser Arg Ser Tyr Arg Ala Pro Glu Val Ile Leu Gly Leu		
	995	1000 1005
Pro Tyr Asp Lys Lys Ile Asp Val Trp Ser Leu Gly Cys Ile Leu		
	1010	1015 1020
Ala Glu Leu Cys Thr Gly Asn Asp Lys Lys Val Asn Pro Cys Leu		
	1025	1030 1035
Asn Ile Glu Leu Leu Leu Gln Val Leu Phe Gln Asn Asp Ser Pro		
	1040	1045 1050
Ala Ser Leu Leu Ala Arg Val Met Gly Ile Val Gly Ser Phe Asp		
	1055	1060 1065
Asn Glu Met Leu Thr Lys Gly Arg Asp Ser His Lys Tyr Phe Thr		
	1070	1075 1080
Lys Asn Arg Met Leu Tyr Glu Arg Asn Gln Glu Ser Asn Arg Leu		
	1085	1090 1095
Glu Tyr Leu Ile Pro Lys Arg Thr Ser Leu Arg His Arg Leu Pro		
	1100	1105 1110
Met Gly Asp Gln Gly Phe Thr Asp Phe Val Ala His Leu Leu Glu		
	1115	1120 1125
Ile Asn Pro Lys Lys Arg Pro Ser Ala Ala Glu Ala Leu Lys His		
	1130	1135 1140
Pro Trp Leu Ser Tyr Pro Tyr Glu Pro Ile Ser Ala		
	1145	1150 1155

&lt;210&gt; SEQ ID NO 37

&lt;211&gt; LENGTH: 654

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Arabidopsis thaliana

&lt;220&gt; FEATURE:

<223> OTHER INFORMATION: receptor-like protein kinase, kinase with  
gatekeeper Cys

&lt;400&gt; SEQUENCE: 37

Met Met Gln Phe His Phe Gln Phe Tyr Val Gly Pro Val Phe Thr Leu
1 5 10 15

Arg Pro Ser Lys Gly Phe Leu Ser Thr Cys Leu Val Ser Phe Leu Phe
20 25 30

Val Thr Thr Thr Phe Cys Ser Tyr Ala Ile Ala Asp Leu Asn Ser Asp
35 40 45

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Arg	Gln	Ala	Leu	Leu	Ala	Phe	Ala	Ala	Ser	Val	Pro	His	Leu	Arg	Arg
50						55					60				
Leu	Asn	Trp	Asn	Ser	Thr	Asn	His	Ile	Cys	Lys	Ser	Trp	Val	Gly	Val
65					70				75					80	
Thr	Cys	Thr	Ser	Asp	Gly	Thr	Ser	Val	His	Ala	Leu	Arg	Leu	Pro	Gly
			85					90					95		
Ile	Gly	Leu	Leu	Gly	Pro	Ile	Pro	Pro	Asn	Thr	Leu	Gly	Lys	Leu	Glu
		100						105				110			
Ser	Leu	Arg	Ile	Leu	Ser	Leu	Arg	Ser	Asn	Leu	Leu	Ser	Gly	Asn	Leu
	115						120					125			
Pro	Pro	Asp	Ile	His	Ser	Leu	Pro	Ser	Leu	Asp	Tyr	Ile	Tyr	Leu	Gln
	130					135					140				
His	Asn	Asn	Phe	Ser	Gly	Glu	Val	Pro	Ser	Phe	Val	Ser	Arg	Gln	Leu
145					150					155					160
Asn	Ile	Leu	Asp	Leu	Ser	Phe	Asn	Ser	Phe	Thr	Gly	Lys	Ile	Pro	Ala
			165					170						175	
Thr	Phe	Gln	Asn	Leu	Lys	Gln	Leu	Thr	Gly	Leu	Ser	Leu	Gln	Asn	Asn
		180						185					190		
Lys	Leu	Ser	Gly	Pro	Val	Pro	Asn	Leu	Asp	Thr	Val	Ser	Leu	Arg	Arg
	195						200					205			
Leu	Asn	Leu	Ser	Asn	Asn	His	Leu	Asn	Gly	Ser	Ile	Pro	Ser	Ala	Leu
	210					215					220				
Gly	Gly	Phe	Pro	Ser	Ser	Ser	Phe	Ser	Gly	Asn	Thr	Leu	Leu	Cys	Gly
225					230					235					240
Leu	Pro	Leu	Gln	Pro	Cys	Ala	Thr	Ser	Ser	Pro	Pro	Pro	Ser	Leu	Thr
			245						250					255	
Pro	His	Ile	Ser	Thr	Pro	Pro	Leu	Pro	Pro	Phe	Pro	His	Lys	Glu	Gly
		260						265					270		
Ser	Lys	Arg	Lys	Leu	His	Val	Ser	Thr	Ile	Ile	Pro	Ile	Ala	Ala	Gly
	275						280					285			
Gly	Ala	Ala	Leu	Leu	Leu	Leu	Ile	Thr	Val	Ile	Ile	Leu	Cys	Cys	Cys
	290					295					300				
Ile	Lys	Lys	Lys	Asp	Lys	Arg	Glu	Asp	Ser	Ile	Val	Lys	Val	Lys	Thr
305					310					315					320
Leu	Thr	Glu	Lys	Ala	Lys	Gln	Glu	Phe	Gly	Ser	Gly	Val	Gln	Glu	Pro
			325						330					335	
Glu	Lys	Asn	Lys	Leu	Val	Phe	Phe	Asn	Gly	Cys	Ser	Tyr	Asn	Phe	Asp
		340						345					350		
Leu	Glu	Asp	Leu	Leu	Arg	Ala	Ser	Ala	Glu	Val	Leu	Gly	Lys	Gly	Ser
	355					360						365			
Tyr	Gly	Thr	Ala	Tyr	Lys	Ala	Val	Leu	Glu	Glu	Ser	Thr	Thr	Val	Val
	370					375					380				
Val	Lys	Arg	Leu	Lys	Glu	Val	Ala	Ala	Gly	Lys	Arg	Glu	Phe	Glu	Gln
385					390					395					400
Gln	Met	Glu	Ile	Ile	Ser	Arg	Val	Gly	Asn	His	Pro	Ser	Val	Val	Pro
			405						410					415	
Leu	Arg	Ala	Tyr	Tyr	Tyr	Ser	Lys	Asp	Glu	Lys	Leu	Met	Val	Cys	Asp
		420						425					430		
Tyr	Tyr	Pro	Ala	Gly	Asn	Leu	Ser	Ser	Leu	Leu	His	Gly	Asn	Arg	Gly
		435						440				445			
Ser	Glu	Lys	Thr	Pro	Leu	Asp	Trp	Asp	Ser	Arg	Val	Lys	Ile	Thr	Leu
	450					455					460				
Ser	Ala	Ala	Lys	Gly	Ile	Ala	His	Leu	His	Ala	Ala	Gly	Gly	Pro	Lys

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465	470	475	480
Phe Ser His Gly Asn Ile Lys Ser Ser Asn Val Ile Met Lys Gln Glu			
	485	490	495
Ser Asp Ala Cys Ile Ser Asp Phe Gly Leu Thr Pro Leu Met Ala Val			
	500	505	510
Pro Ile Ala Pro Met Arg Gly Ala Gly Tyr Arg Ala Pro Glu Val Met			
	515	520	525
Glu Thr Arg Lys His Thr His Lys Ser Asp Val Tyr Ser Phe Gly Val			
	530	535	540
Leu Ile Leu Glu Met Leu Thr Gly Lys Ser Pro Val Gln Ser Pro Ser			
	545	550	555
Arg Asp Asp Met Val Asp Leu Pro Arg Trp Val Gln Ser Val Val Arg			
	565	570	575
Glu Glu Trp Thr Ser Glu Val Phe Asp Ile Glu Leu Met Arg Phe Gln			
	580	585	590
Asn Ile Glu Glu Glu Met Val Gln Met Leu Gln Ile Ala Met Ala Cys			
	595	600	605
Val Ala Gln Val Pro Glu Val Arg Pro Thr Met Asp Asp Val Val Arg			
	610	615	620
Met Ile Glu Glu Ile Arg Val Ser Asp Ser Glu Thr Thr Arg Pro Ser			
	625	630	635
Ser Asp Asp Asn Ser Lys Pro Lys Asp Ser Asn Val Gln Val			
	645	650	

&lt;210&gt; SEQ ID NO 38

&lt;211&gt; LENGTH: 420

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Mus musculus

&lt;220&gt; FEATURE:

<223> OTHER INFORMATION: MAPK/MAK/MRK overlapping kinase, MOK protein  
kinase, T/STK 30, renal tumor antigen, RAGE1, kinase with  
gatekeeper Cys

&lt;400&gt; SEQUENCE: 38

Met Lys Asn Tyr Lys Ala Ile Gly Lys Ile Gly Glu Gly Thr Phe Ser			
1	5	10	15
Glu Val Met Lys Met Gln Ser Leu Arg Asp Gly Asn Tyr Tyr Ala Cys			
	20	25	30
Lys Gln Met Lys Gln His Phe Glu Ser Ile Glu Gln Val Asn Ser Leu			
	35	40	45
Arg Glu Ile Gln Ala Leu Arg Arg Leu Asn Pro His Pro Asn Ile Leu			
	50	55	60
Ala Leu His Glu Val Val Phe Asp Arg Lys Ser Gly Ser Leu Ala Leu			
	65	70	75
Ile Cys Glu Leu Met Asp Met Asn Ile Tyr Glu Leu Ile Arg Gly Arg			
	85	90	95
Arg His Pro Leu Ser Glu Lys Lys Ile Met Leu Tyr Met Tyr Gln Leu			
	100	105	110
Cys Lys Ser Leu Asp His Met His Arg Asn Gly Ile Phe His Arg Asp			
	115	120	125
Val Lys Pro Glu Asn Ile Leu Val Lys Gln Asp Val Leu Lys Leu Gly			
	130	135	140
Asp Phe Gly Ser Cys Arg Ser Val Tyr Ser Lys Gln Pro Tyr Thr Glu			
	145	150	155
Tyr Ile Ser Thr Arg Trp Tyr Arg Ala Pro Glu Cys Leu Leu Thr Asp			
	165	170	175

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Gly Phe Tyr Thr Tyr Lys Met Asp Leu Trp Ser Ala Gly Cys Val Phe  
 180 185 190  
 Tyr Glu Ile Ala Ser Leu Gln Pro Leu Phe Pro Gly Val Asn Glu Leu  
 195 200 205  
 Asp Gln Ile Ser Lys Ile His Asp Val Ile Gly Thr Pro Cys Gln Lys  
 210 215 220  
 Thr Leu Thr Lys Phe Lys Gln Ser Arg Ala Met Ser Phe Asp Phe Pro  
 225 230 235 240  
 Phe Lys Lys Gly Ser Gly Ile Pro Leu Leu Thr Ala Asn Leu Ser Pro  
 245 250 255  
 Gln Cys Leu Ser Leu Leu His Ala Met Val Ala Tyr Asp Pro Asp Glu  
 260 265 270  
 Arg Ile Ala Ala His Gln Ala Leu Gln His Pro Tyr Phe Gln Val Gln  
 275 280 285  
 Arg Ala Ala Glu Thr Gln Thr Leu Ala Lys His Arg Arg Ala Phe Cys  
 290 295 300  
 Pro Lys Phe Ser Met Val Pro Glu Ser Ser Ser His Asn Trp Ser Phe  
 305 310 315 320  
 Ser Gln Glu Gly Arg Lys Gln Lys Gln Ser Leu Arg His Glu Glu Gly  
 325 330 335  
 His Ala Arg Arg Gln Gly Pro Thr Ser Leu Met Glu Leu Pro Lys Leu  
 340 345 350  
 Arg Leu Ser Gly Met Thr Lys Leu Ser Ser Cys Ser Ser Pro Ala Leu  
 355 360 365  
 Arg Ser Val Leu Gly Thr Gly Ala Asn Gly Lys Val Pro Val Leu Arg  
 370 375 380  
 Pro Leu Lys Cys Ala Ala Val Asn Lys Lys Thr Asp Thr Gln Lys Asp  
 385 390 395 400  
 Ile Lys Pro His Leu Lys His Tyr His Leu Pro Thr Ile Asn Arg Lys  
 405 410 415  
 Gly Gly Glu Tyr  
 420

&lt;210&gt; SEQ ID NO 39

&lt;211&gt; LENGTH: 344

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Carassius auratus

&lt;220&gt; FEATURE:

 <223> OTHER INFORMATION: cyclin-dependent kinase 7 (CDK7), 40kDa protein  
 kinase, CDC2/CDK2, 4-activating kinase, cell division protein  
 kinase 7, P40 M015, kinase with gatekeeper Cys

&lt;400&gt; SEQUENCE: 39

Met Ala Leu Asp Val Lys Ser Arg Ala Lys Leu Tyr Glu Lys Leu Asp  
 1 5 10 15  
 Phe Leu Gly Glu Gly Gln Phe Ala Thr Val Tyr Lys Ala Arg Asp Lys  
 20 25 30  
 Thr Thr Asn Thr Ile Val Ala Ile Lys Lys Ile Lys Val Gly His Arg  
 35 40 45  
 Thr Glu Ala Lys Asp Gly Ile Asn Arg Thr Ala Leu Arg Glu Ile Lys  
 50 55 60  
 Leu Leu Gln Glu Leu Ser His Pro Asn Ile Ile Gly Leu Leu Asp Ala  
 65 70 75 80  
 Phe Gly His Lys Ser Asn Ile Ser Leu Leu Cys Phe Met Glu Thr Asp  
 85 90 95

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Leu Glu Val Ile Ile Lys Asp Thr Ser Leu Val Leu Thr Pro Ala Asn
    100                      105                      110

Ile Lys Ala Tyr Ile Leu Met Ser Leu Gln Gly Leu Glu Tyr Met His
    115                      120                      125

Asn His Trp Ile Leu His Arg Asp Leu Lys Pro Asn Asn Leu Leu Leu
    130                      135                      140

Asp Glu Asn Gly Val Leu Lys Leu Ala Asp Phe Gly Leu Ala Lys Ala
    145                      150                      155                      160

Phe Gly Ser Pro Asn Arg Val Tyr Thr His Gln Val Val Thr Arg Trp
    165                      170                      175

Tyr Arg Ala Pro Glu Leu Leu Phe Gly Ala Arg Met Tyr Gly Val Gly
    180                      185                      190

Val Asp Met Trp Ala Val Gly Ser Ile Leu Ala Glu Leu Leu Leu Arg
    195                      200                      205

Val Pro Phe Leu Ala Gly Asp Ser Asp Leu Asp Gln Leu Thr Gly Ile
    210                      215                      220

Phe Glu Ala Leu Gly Thr Pro Thr Glu Glu Thr Trp Pro Gly Met Ser
    225                      230                      235                      240

Asn Leu Pro Asp Tyr Val Ser Phe Lys Leu Phe Pro Gly Thr Pro Leu
    245                      250                      255

Glu His Ile Phe Ser Ala Ala Gly Asp Asp Leu Leu Glu Leu Leu Lys
    260                      265                      270

Gly Leu Phe Thr Phe Asn Pro Cys Thr Arg Thr Thr Ala Ser Gln Ala
    275                      280                      285

Leu Lys Met Arg Tyr Phe Ser Ile Arg Pro Gly Pro Thr Pro Gly Pro
    290                      295                      300

Gln Leu Pro Arg Pro Asn Ser Ser Thr Glu Ala Leu Lys Glu Lys Glu
    305                      310                      315                      320

Asn Leu Leu Ile Gly Ile Lys Arg Lys Arg Asp Ser Ile Glu Gln Gly
    325                      330                      335

Thr Leu Lys Lys Lys Leu Val Phe
    340

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&lt;210&gt; SEQ ID NO 40

&lt;211&gt; LENGTH: 134

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Capsicum annuum

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: protein kinase homolog D3, kinase with gatekeeper Cys

&lt;400&gt; SEQUENCE: 40

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Thr Ser Gly Cys Glu Val Ala Leu Lys Arg Phe Lys Asn Cys Ser Ala
  1      5      10      15

Gly Gly Asp Ser Ser Phe Ile His Glu Leu Glu Val Ile Ala Ser Val
  20      25      30

Arg His Val Asn Leu Leu Gly Leu Arg Gly Tyr Cys Thr Ala Thr Phe
  35      40      45

Pro Leu Glu Gly His Gln Arg Ile Ile Val Cys Asp Leu Val Lys His
  50      55      60

Gly Ser Leu Tyr Asp His Leu Phe Gly Leu Arg Cys Asn Lys Leu Ser
  65      70      75      80

Trp Pro Ile Arg Gln Arg Ile Ala Ile Gly Thr Ala Arg Gly Leu Ala
  85      90      95

Tyr Leu His Tyr Gly Ala Gln Pro Ala Ile Ile His Arg Asp Ile Lys
  100     105     110

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Ser Ser Asn Ile Leu Leu Asp Glu Asn Phe Glu Pro Lys Val Ala Asp  
 115 120 125

Phe Gly Leu Ala Lys Leu  
 130

<210> SEQ ID NO 41

<211> LENGTH: 983

<212> TYPE: PRT

<213> ORGANISM: Leishmania major

<220> FEATURE:

<223> OTHER INFORMATION: possible serine/threonine kinase, kinase with  
 gatekeeper Cys

<400> SEQUENCE: 41

Met Glu Glu Tyr Thr Ile Lys Arg Lys Ile Gly Asp Gly Ala Gln Gly  
 1 5 10 15

Val Val Tyr Glu Val Glu His Arg Thr Ser Lys Thr Ser Tyr Ala Met  
 20 25 30

Lys Val Ile Cys Cys Thr Asp Gln Glu Gln Val Asn Met Ala Leu Lys  
 35 40 45

Glu Ile Lys Val Leu Leu Gln Leu Arg His Pro Ser Ile Val Ser Tyr  
 50 55 60

Val Asp Phe Phe Leu Val Phe Asn Ser Val Lys Leu Arg Arg Glu Phe  
 65 70 75 80

Ala Ala Gln Ser Glu Gly Ala Cys Gly Ser Gly Gly Cys Gly Asn Gly  
 85 90 95

Gln Gln Arg Glu Gln Asp Ser Leu Phe Leu Cys Ser Leu Ser Asn Ser  
 100 105 110

Glu Leu Asp His Gly Cys Ala Ala Asp Ser Gly Trp Lys Pro Ala Ala  
 115 120 125

Ala Glu Ala Ser Ser Asn Ala Pro Thr Gly Lys Pro Gln Ser Gly Ala  
 130 135 140

Thr Arg Val Val Pro Thr Ser Leu Leu Ser Lys His Arg Gln Gln Ala  
 145 150 155 160

Gly Ala His Trp Leu Gly Glu Glu Glu Ile Ala Val Cys Leu Val Met  
 165 170 175

Glu Leu Cys Ser Asn Gly Asp Met Gln Gly Leu Val Arg Glu Thr Arg  
 180 185 190

Gln Glu Phe Met Lys Thr Gly Ser His Ser Ile Ala Glu Ala Gln Ala  
 195 200 205

Val Ser Trp Leu Glu Gln Ala Ala Ala Ala Leu Gln Phe Ile His Asn  
 210 215 220

Lys Gly Phe Leu His Arg Asp Leu Lys Pro Thr Asn Ile Phe Phe Asp  
 225 230 235 240

Glu Tyr Lys Asn Ile Lys Val Gly Asp Phe Gly Leu Ala Ala Thr Val  
 245 250 255

Gly Leu Gly Arg Asn Ser Ala Val Gly Thr Pro Tyr Tyr Leu Ala Pro  
 260 265 270

Glu Arg Met Leu Gln Gln Arg Tyr Asp Gly Lys Val Asp Ile Trp Gly  
 275 280 285

Leu Gly Val Val Leu Leu Glu Leu Leu Thr Leu Arg Glu Gln Pro Ile  
 290 295 300

Asn Ser Met Leu Leu Glu Asn Pro Lys Val Val Asp Thr Val Ile Pro  
 305 310 315 320

Gln Ile Thr Lys Met Gly Tyr Ser Thr Lys Leu Ala Thr Leu Leu Arg

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325								330					335				
Asp	Met	Leu	Gln	Arg	Gln	Pro	Gln	Asp	Arg	Pro	Thr	Pro	Ser	Ser	Ile		
			340					345					350				
Leu	His	Arg	Leu	Ala	Ser	Ile	Thr	Ala	Thr	Ser	Pro	His	Pro	Gly	Met		
			355				360					365					
Ser	Ala	Thr	Leu	Phe	Ala	Gly	Met	Ser	Cys	Pro	Lys	Leu	Thr	Glu	Ala		
			370			375					380						
Leu	Cys	Asp	Val	Cys	Glu	Val	Glu	Val	Ala	Gly	Val	Met	Cys	Ser	Ser		
385					390					395					400		
Cys	Lys	Ala	Ala	Phe	Cys	Ala	Gly	Cys	Asp	Arg	Ala	Arg	His	Arg	His		
				405					410					415			
His	Ser	Arg	Gln	Ser	His	Asp	Arg	Thr	Asn	Met	Ser	Ser	Ile	Val	Asn		
			420					425					430				
Ser	Met	Asn	Gly	Ala	Ser	Ser	Leu	Pro	Leu	Ser	Ala	Thr	Pro	Met	Gln		
			435				440					445					
Gln	Gln	Gln	Gln	Gln	Gln	Lys	Thr	Leu	Ser	Phe	Ser	Arg	Gly	Pro	Ser		
		450				455						460					
Pro	Ala	Asn	Thr	Ser	Asp	Gln	Thr	Arg	Ala	Ser	Met	Gln	Asn	Ile	Val		
465					470					475					480		
Val	Phe	Pro	Ser	Ser	Asn	Ser	Ser	His	Ser	Arg	Thr	Leu	Pro	Arg	Glu		
					485					490				495			
Arg	Glu	Met	Asn	Ser	Arg	Thr	Phe	Thr	Arg	Phe	Gln	Leu	Ala	Leu	Pro		
			500					505						510			
Gly	Arg	Ser	Val	Ser	Met	Ser	Asp	Phe	Ser	Met	Thr	Gln	Gly	Leu	Gln		
			515				520					525					
Gly	Pro	Arg	Asp	Gly	Ser	Gly	Ile	Asn	Ala	Ala	Val	Ala	Glu	Thr	Val		
			530			535					540						
Leu	Arg	Val	Pro	Asp	Asp	Val	Pro	Ser	Leu	Ala	Gln	Ala	Leu	Arg	Val		
545					550					555					560		
Val	Glu	Ser	Met	Pro	His	Ile	Arg	Lys	Ile	Leu	Val	Ala	Gly	Asn	Thr		
				565				570						575			
Thr	His	Thr	Val	Pro	Leu	Val	Leu	Thr	Ser	Arg	Leu	Pro	Asp	Ser	Ile		
			580					585					590				
Lys	Leu	Val	Gly	Glu	Ser	Pro	Pro	Pro	Met	Leu	Glu	Val	Ala	Asp	Ser		
			595				600					605					
Pro	Phe	Ala	Leu	His	Cys	His	Ser	Gly	Arg	Gly	Ser	Val	Glu	Asn	Phe		
			610			615					620						
Ile	Leu	Arg	His	Val	Gly	Arg	Phe	Cys	Phe	Lys	Leu	Leu	Lys	Leu	Asp		
625					630					635					640		
Thr	Asn	Leu	His	Gln	Thr	Asp	Ala	Asn	Ala	Met	Thr	Ser	Ala	Pro	Ala		
				645				650						655			
Lys	Lys	Pro	Ser	Arg	Pro	Thr	Ala	Val	Ser	Ile	Thr	Gly	Gly	Glu	Trp		
			660					665					670				
Arg	Leu	His	Lys	Cys	Arg	Ile	Ser	Cys	Val	Glu	Gly	Ser	Gly	Val	Thr		
			675				680					685					
Val	Gly	Gly	Ser	Lys	His	Thr	Pro	Ser	Ser	Ala	Thr	Asn	Gly	Gln	Asn		
			690			695					700						
Pro	Ser	Ala	Thr	Gly	Ala	Arg	Ser	Ser	Arg	Pro	Pro	Gln	Ser	Pro	Ser		
705					710					715					720		
Leu	Val	Ala	Arg	Ser	Ser	Leu	Val	Asn	Gly	Ala	Asp	Glu	Gly	Ala	Glu		
				725				730						735			
Asp	Ala	Asp	Val	Met	Ser	Met	Glu	Pro	Ile	Ile	Thr	Lys	Cys	Ser	Phe		
			740					745					750				

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Ile Asp Val Thr Ala Ala Gly Ile Val Val Met Glu Lys Ser Arg Gly
   755                               760               765
Leu Tyr Glu Gly Asn Thr Phe Ser Gly Cys Gly Phe Ala Ala Phe Leu
   770                               775               780
Leu Arg Lys Asp Ala Thr Pro Arg Ile Arg Ala Asn His Ile Thr Asp
   785                               790               795               800
Gly Ala Glu Ala Gly Ile Phe Cys Gln Asp Ala Ser Gly Leu Met Glu
                   805                               810               815
Tyr Asn Val Ile Ala Gln Asn Ala Gly Cys Gly Ile Val Val Lys Gly
                   820                               825               830
Ala Ser Ala Val Pro Val Ile Arg Lys Asn Arg Val Leu Ser Asn Val
                   835                               840               845
Gln Ala Gly Val Phe Cys Cys Asp Lys Ala Ala Pro Phe Val Ser Asp
   850                               855               860
Asn Glu Ile Arg Gln Asn Gly Lys Ala Gly Val Leu Val Lys Thr Thr
   865                               870               875               880
Ala Ala Pro Lys Ile Thr Arg Asn Val Ile Glu Ser Gly Lys Glu Ala
                   885                               890               895
Gly Ile Tyr Ile Phe Glu Lys Gly Ala Gly Ile Ile Glu Glu Asn Arg
                   900                               905               910
Ile Arg Gly Asn Gln Asn Ala Gly Leu Leu Val Thr Thr Gly Gly Asn
   915                               920               925
Pro His Val Ile His Asn Thr Ile Thr Lys Asn Ala Tyr Glu Gly Ile
   930                               935               940
Trp Val Cys Lys His Gly Gly Gly Thr Phe Cys Asp Asn Asp Leu Arg
   945                               950               955               960
Gly Asn Thr Lys Gly Ala Lys Asp Ile Glu Ala Asp Ser Arg Val Thr
                   965                               970               975
Trp Val Gly Asn Val Glu Gln
                   980

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&lt;210&gt; SEQ ID NO 42

&lt;211&gt; LENGTH: 419

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Homo sapiens

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: MOK protein kinase, kinase with gatekeeper Cys

&lt;400&gt; SEQUENCE: 42

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Met Lys Asn Tyr Lys Ala Ile Gly Lys Ile Gly Glu Gly Thr Phe Ser
 1           5           10           15
Glu Val Met Lys Met Gln Ser Leu Arg Asp Gly Asn Tyr Tyr Ala Cys
 20           25           30
Lys Gln Met Lys Gln Arg Phe Glu Ser Ile Glu Gln Val Asn Asn Leu
 35           40           45
Arg Glu Ile Gln Ala Leu Arg Arg Leu Asn Pro His Pro Asn Ile Leu
 50           55           60
Met Leu His Glu Val Val Phe Asp Arg Lys Ser Gly Ser Leu Ala Leu
 65           70           75           80
Ile Cys Glu Leu Met Asp Met Asn Ile Tyr Glu Leu Ile Arg Gly Arg
 85           90           95
Arg Tyr Pro Leu Ser Glu Lys Lys Ile Met His Tyr Met Tyr Gln Leu
100          105          110
Cys Lys Ser Leu Asp His Ile His Arg Asn Gly Ile Phe His Arg Asp
115          120          125

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Val Lys Pro Glu Asn Ile Leu Ile Lys Gln Asp Val Leu Lys Leu Gly  
 130 135 140  
 Asp Phe Gly Ser Cys Arg Ser Val Tyr Ser Lys Gln Pro Tyr Thr Glu  
 145 150 155 160  
 Tyr Ile Ser Thr Arg Trp Tyr Arg Ala Pro Glu Cys Leu Leu Thr Asp  
 165 170 175  
 Gly Phe Tyr Thr Tyr Lys Met Asp Leu Trp Ser Ala Gly Cys Val Phe  
 180 185 190  
 Tyr Glu Ile Ala Ser Leu Gln Pro Leu Phe Pro Gly Val Asn Glu Leu  
 195 200 205  
 Asp Gln Ile Ser Lys Ile His Asp Val Ile Gly Thr Pro Ala Gln Lys  
 210 215 220  
 Ile Leu Thr Lys Phe Lys Gln Ser Arg Ala Met Asn Phe Asp Phe Pro  
 225 230 235 240  
 Phe Lys Lys Gly Ser Gly Ile Pro Leu Leu Thr Thr Asn Leu Ser Pro  
 245 250 255  
 Gln Cys Leu Ser Leu Leu His Ala Met Val Ala Tyr Asp Pro Asp Glu  
 260 265 270  
 Arg Ile Ala Ala His Gln Ala Leu Gln His Pro Tyr Phe Gln Glu Gln  
 275 280 285  
 Arg Lys Thr Glu Lys Arg Ala Leu Gly Ser His Arg Lys Ala Gly Phe  
 290 295 300  
 Pro Glu His Pro Val Ala Pro Glu Pro Leu Ser Asn Ser Cys Gln Ile  
 305 310 315 320  
 Ser Lys Glu Gly Arg Lys Gln Lys Gln Ser Leu Lys Gln Glu Glu Asp  
 325 330 335  
 Arg Pro Lys Arg Arg Gly Pro Ala Tyr Val Met Glu Leu Pro Lys Leu  
 340 345 350  
 Lys Leu Ser Gly Val Val Arg Leu Ser Ser Tyr Ser Ser Pro Thr Leu  
 355 360 365  
 Gln Ser Val Leu Gly Ser Gly Thr Asn Gly Arg Val Pro Val Leu Arg  
 370 375 380  
 Pro Leu Lys Cys Ile Pro Ala Ser Lys Lys Thr Asp Pro Gln Lys Asp  
 385 390 395 400  
 Leu Lys Pro Ala Pro Gln Gln Cys Arg Leu Pro Thr Ile Val Arg Lys  
 405 410 415  
 Gly Gly Arg

&lt;210&gt; SEQ ID NO 43

&lt;211&gt; LENGTH: 278

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Trichomonas vaginalis

&lt;220&gt; FEATURE:

<223> OTHER INFORMATION: probable protein kinase cdc2/cdc28-related,  
kinase with gatekeeper Cys

&lt;400&gt; SEQUENCE: 43

Met Asp Leu Ser Ala Tyr His Lys Asp Met Lys Leu Gly Glu Gly Thr  
 1 5 10 15  
 Tyr Gly Ser Val Phe Arg Ala Thr His Ile Pro Thr Asp Gln Pro Val  
 20 25 30  
 Val Leu Lys Leu Val Arg Met Asp Leu Glu Glu Asp Gly Ile Pro Pro  
 35 40 45  
 Ser Ser Val Arg Glu Val Cys Ile Leu Lys Ser Leu Asn His Pro Asn  
 50 55 60

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Ile Leu His Phe Arg Glu Val Ile Cys Lys Asp Ser Lys Ile Ile Met  
 65 70 75 80  
 Val Cys Glu Phe Met Asp Met Asp Leu Lys Asn Phe Leu Ser Lys Arg  
 85 90 95  
 Arg Met Asn Pro Asp Leu Leu Arg Ser Tyr Ala Phe Gln Leu Leu Cys  
 100 105 110  
 Gly Thr Tyr Tyr Leu His Arg Ile Gly Ile Val His Arg Asp Ile Lys  
 115 120 125  
 Pro Glu Asn Ile Leu Ile Asp Arg Asn Gly Leu Leu Lys Leu Gly Asp  
 130 135 140  
 Phe Gly Thr Ala Ala Tyr Cys Phe His Pro Ile Pro Tyr Asp Ile Glu  
 145 150 155 160  
 Glu Ile Lys Thr Pro Trp Tyr Leu Ala Pro Glu Ile Leu Ile Asn Ala  
 165 170 175  
 Pro Ala His Gly Thr Glu Ile Asp Ile Trp Ser Ile Gly Cys Val Ile  
 180 185 190  
 Ala Glu Met Ala Arg Gly Asn Leu Phe Met Gly Asp Ser Gln Val Asp  
 195 200 205  
 Gln Leu Ile Lys Ile Thr Glu Val Leu Gly Ile Pro Ser Glu Glu Asp  
 210 215 220  
 Tyr Pro Asp Phe Tyr Lys Tyr Lys Ile Asn Asn Met Pro Cys Met Lys  
 225 230 235 240  
 Lys Glu Lys Pro Asp Phe Asn Ser Phe Phe Pro Gly Val Asp Pro Glu  
 245 250 255  
 Leu Val Asp Leu Ile Ser Lys Met Leu Gln Met Asn Pro Glu His Arg  
 260 265 270  
 Ile Asn Ala Gln Thr His  
 275

<210> SEQ ID NO 44  
 <211> LENGTH: 2485  
 <212> TYPE: PRT  
 <213> ORGANISM: Plasmodium falciparum  
 <220> FEATURE:  
 <223> OTHER INFORMATION: putative protein kinase, Ser/Thr protein  
 kinase, kinase with gatekeeper Cys

<400> SEQUENCE: 44

Met Phe Ser Val Glu Leu Glu Asn Arg Ser Gly Tyr Lys Lys Arg Lys  
 1 5 10 15  
 Lys Lys Lys Trp Asn Asn Lys Ser Thr Gly Gln Asp Lys Phe Thr Asn  
 20 25 30  
 Lys Asp Ile Ile Ser Glu Glu Lys Glu Glu Gly Leu Asp Ile Glu Cys  
 35 40 45  
 Gly His Asn Ile Leu Gly Asp Val Gln Tyr Asp Gly Thr Tyr Asn Ile  
 50 55 60  
 Asn Glu Gln Val Lys Lys Asn Ser Leu Phe Tyr Phe Lys Cys Lys Glu  
 65 70 75 80  
 Glu Ile Asn Leu Lys Asp Gly Asn Ile Ile Leu Asp Asp Lys Asn Arg  
 85 90 95  
 Lys Val Asp Asp Ile Asn Ile Thr Gly Asp Asp Lys Asn Ile Lys Val  
 100 105 110  
 Asp Asp Lys Asn Ile Lys Val Asp Asp Lys Asn Ile Thr Gly Glu Asp  
 115 120 125  
 Lys Asn Ile Thr Gly Glu Asp Lys Asn Ile Thr Gly Asp Asp Lys Asn

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130	135	140
Ile Ile Phe Asp Val Asp Glu Ile Leu Ile His Gln His Asn Thr Ser		
145	150	155 160
Asn Ser Asn Ile Tyr Ile Asn Cys Asn Asp Asn Asn Asp Ile Arg		
	165	170 175
Asn Ser Ser Asn Val Gln His Tyr Tyr Asn Asp Lys Ile Lys Glu Asn		
	180	185 190
Ile Asn Lys Gln Asn Lys Lys Tyr Val Leu Ile Asn Asp Tyr Ile Asn		
	195	200 205
Asn Lys Tyr Ile Leu Ser Lys Asn Lys Thr Cys Lys Ile Asn Lys Gly		
	210	215 220
Lys Lys Leu Ile Lys Lys Lys Lys Val Asn Asn Ile Ser Arg Arg Arg		
	225 230	235 240
Asn His Ile Leu Tyr Lys Cys Arg Asn Lys Leu Tyr Asn Gly Asn Val		
	245	250 255
Phe Ser Asp Asp Ile Ile Lys Ser Glu Val Asn Val Cys Asn Ser Leu		
	260	265 270
Thr Val Leu His Lys Asn Tyr Asn Ile Asn Met Asp Asn Tyr Leu Asp		
	275	280 285
Asp Asn Ile His Thr Asn Asn Ser Asn Ile Tyr Asp Ile Asn Tyr Thr		
	290	295 300
Asn Glu Asn Val Ile Asn Ser Thr Cys Arg Tyr Tyr Pro Ile Gly Asn		
	305 310	315 320
Asn Asn Thr Leu Ser Lys Asp Glu Val Thr Lys Ser Ser Ser Lys Ile		
	325	330 335
Asn Ser Leu Ser Tyr Phe Asp Asp Ile Ile Asn Val Asn Lys Asn Asp		
	340	345 350
Ile Pro Ile Leu His Asp Lys Glu Asn Ile Asn Ile Ile Ser Asn Lys		
	355	360 365
Glu Ser Cys His Lys Asp Glu Lys Glu Glu Glu Lys Tyr Ile Met Tyr		
	370	375 380
Asn Ser Asn Leu Val Glu Glu Lys Lys Gln Lys Lys Met Ile Trp Asn		
	385 390	395 400
Ser Leu Asn Val Leu Pro Ile Asp Ile Leu Leu Lys Asn Gly His Asp		
	405	410 415
Glu Ile Asn Lys Glu Ile Cys Lys Lys Lys Lys Lys Ser Phe Phe Ser		
	420	425 430
Gln Asn Asp Ile Lys Ser Lys Met Leu Tyr Asn Asn Lys Ser Tyr Ser		
	435	440 445
Lys Ser Glu Lys Val Leu Tyr Thr Asn Asn Lys Asn Ser Asn Thr Phe		
	450	455 460
Ile Pro Ile Phe Phe Leu Asn Lys Val Gly Asp Lys Phe Lys Asn Ser		
	465 470	475 480
Glu Asn Ile Tyr Asp Met Tyr Asn Asn Lys Lys Asn Val Tyr Ile His		
	485	490 495
Asp Lys Lys Ile Tyr Thr Asn Met Tyr Ser Asn Lys Leu Lys Gln Lys		
	500	505 510
His Tyr Tyr Ser Thr Ser Asn Ile Asn Leu Leu Tyr Asn Asn Ile Gly		
	515	520 525
Lys Val Leu Asp Asn Gly Leu His Leu Ser Asn Asn Met Tyr Cys Arg		
	530	535 540
Leu Asn Ser Asn Pro Pro Tyr Lys Ser Ile Ser Leu Ile Asn Asn Asn		
	545 550	555 560

Val	Phe	Phe	Tyr	Lys	Lys	Arg	Lys	Ser	Asn	Ser	Asn	Asn	Asn	Asn	565
Asn	Asn	Asn	Ile	Ser	Ser	Ser	Ser	Ser	Ser	Ser	Ser	Lys	Lys	Asn	His
Val	Ile	Ile	Asn	Lys	Lys	Ile	Ser	Ser	Tyr	Asn	Ile	His	Tyr	Lys	Glu
Arg	Lys	Asp	Ser	Phe	Lys	Glu	Asn	Phe	Leu	Phe	Phe	Lys	Glu	Lys	Ile
Leu	Pro	Ser	Lys	Lys	Asp	Thr	Cys	Val	Phe	Asn	Glu	Arg	Gln	Lys	Asp
Leu	Phe	Glu	Lys	Ser	Asn	Glu	His	Ile	Lys	Cys	Val	Ser	Ser	Phe	Asn
Asn	Thr	Ser	Asp	Asp	Ile	Ser	Ser	His	Ser	Ser	Val	Asn	Lys	Lys	Glu
Pro	Phe	Phe	Ala	Leu	Lys	Asn	Asn	Ser	Ile	Arg	His	Ile	Pro	Lys	Glu
Asn	Asn	Ile	Ile	Tyr	Thr	Ser	Gly	Lys	Ser	Phe	Asn	His	Val	Gln	Asp
Lys	Glu	Lys	Thr	Val	Leu	Leu	Lys	Lys	Lys	Lys	Glu	Ile	Asn	Asp	Lys
Asn	Thr	Phe	Ser	Ser	Cys	Leu	Ile	Asn	His	Asn	Ile	Thr	Thr	Tyr	Thr
Leu	Gln	Asn	Gly	Val	Asn	Lys	Asn	Leu	Asn	Met	Leu	Gly	Ile	Arg	Asp
Ser	Ile	Tyr	Lys	Ile	Asp	Glu	Lys	Asn	Asn	Met	Leu	Lys	Glu	Cys	Tyr
Asn	Gly	Asn	Asn	Asp	Ser	Asn	Asn	Lys	Lys	Lys	Lys	Lys	Lys	Lys	Lys
Leu	Ser	Phe	Ser	Cys	Asp	Ile	Ile	Asn	Asp	Asn	Ile	Thr	Pro	Tyr	Glu
Ser	Asp	Lys	Glu	Lys	Asn	Asn	Ser	Asn	Asn	Ile	Lys	Ser	Met	Asp	Ile
Phe	Asn	Tyr	Val	Lys	Arg	Lys	Ser	Asn	Leu	Tyr	Asn	Asn	Leu	Ser	Ser
Asn	Arg	Asp	Ser	Thr	Val	Asp	Met	His	Asn	Lys	Tyr	Asn	Ser	Glu	Glu
Tyr	Ile	Asn	Ile	Gln	Arg	Thr	Asn	Lys	Ile	Tyr	Glu	Leu	Ser	Asn	Lys
Arg	Ile	Arg	Asn	Tyr	Lys	Leu	Tyr	Ser	Met	Asp	Glu	Ile	Phe	Lys	Val
Ser	Leu	Lys	Glu	Lys	Lys	Tyr	Ile	Asp	Asn	Ile	Ser	Asn	Asn	Met	Glu
Arg	Val	Thr	Tyr	Lys	Asn	Glu	Met	Ile	Asn	Glu	Lys	Ile	Ser	Lys	Met
Asp	Asp	Ile	Leu	Tyr	Pro	Cys	Asp	Lys	Asn	Lys	Ser	Leu	Asn	Met	Ser
Cys	Pro	Val	Ile	Ile	Glu	Asn	Asn	Ile	Ser	Arg	Glu	Glu	Asn	Glu	Lys
Asn	Ser	Ser	Val	Ile	Leu	Asn	Lys	Lys	Lys	Asn	Glu	Asn	Met	Phe	Asn
Cys	Val	Gly	Arg	Leu	His	Cys	His	Met	Gly	Lys	Met	Asn	Asn	Gln	Asp

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Asn Ile Tyr Asp Gln Gly	Asn Ile Lys Lys Asn Glu Glu Glu Ile Thr
980	985 990
Lys His Asp Glu Tyr Ile Ser Arg	Glu Glu Lys Asn Lys Tyr Asn Ser
995	1000 1005
Lys Cys Ile Arg Asn Phe Asp	Asp Tyr Lys Tyr Glu Gln Val Leu
1010	1015 1020
Ser Tyr His Thr Leu Asp Glu	Asp Lys Lys Lys Asn Asp Met Asn
1025	1030 1035
Asn Leu Ile Asp Met Asn Asn	Glu Ala Ile Ile Glu Thr Val Asn
1040	1045 1050
Gly Val Ile Asn Asn Ile Ile	Leu Asp Arg Lys Asp Asn Asn Ser
1055	1060 1065
Arg Lys Asp Met Glu Lys Glu	Met Glu Lys Glu Met Glu Lys Lys
1070	1075 1080
Met Glu Lys Glu Met Glu Lys	Val Met Glu Lys Glu Met Glu Lys
1085	1090 1095
Val Met Glu Lys Glu Val Glu	Lys Glu Leu Lys Asn Glu Met Asn
1100	1105 1110
Asn Arg Met Asn Asn Arg Met	Asn Asn Glu Met Lys Asn Glu Ile
1115	1120 1125
Asn Ile Tyr Lys Asn Asn Glu	Ile Tyr Val Asp Asn Asp Lys Glu
1130	1135 1140
Leu Glu Ile Val Asn Glu Glu	Lys Lys Leu Ile Tyr Pro Phe Asn
1145	1150 1155
Tyr Glu Ser Asp Val His Lys	Asn Met Asn Met Ser Ile Asn Ile
1160	1165 1170
Asn Asn Cys Lys Asp Asp Tyr	Asn Asn Ile Leu Lys Glu Tyr Val
1175	1180 1185
Asp Asn Ser Cys Leu Ala Gln	Lys Glu Glu Asn Ile Phe Arg Pro
1190	1195 1200
Leu Phe Asn Leu Asn Lys Lys	Asp Lys Val Trp Lys Arg Phe Asn
1205	1210 1215
Ile Lys Asn Asn Ile Lys Thr	Ile Ile His Asn Glu Glu Met Lys
1220	1225 1230
Arg Ile Tyr Gln Thr Ile Asn	Lys Asn Val Phe Pro Ile Tyr Asn
1235	1240 1245
Phe Asn Arg Tyr Glu Asn Phe	Leu Ile Asn His Leu Thr Tyr Asn
1250	1255 1260
Phe Pro Lys Asn Asp Leu Phe	Lys Leu Ser Tyr Lys Val Ser Met
1265	1270 1275
Asn Asn Ile Arg Asn Leu Tyr	Ile Ala Asn Lys His Ile Asn Asn
1280	1285 1290
Asn Tyr Asp Tyr Met Asn Lys	Leu Tyr Asn Gln Asn Ile Tyr Thr
1295	1300 1305
Leu Lys Tyr Gln Val Ala Asn	Ile Asp Asn Asp His His Ile Cys
1310	1315 1320
Lys Lys Gly Gly Gly Leu Asp	Tyr Ile Asn Met Asn Ile Ser Lys
1325	1330 1335
Glu Cys Lys Asn Arg Lys Asp	Lys Thr Tyr Leu Asn Lys Ile Phe
1340	1345 1350
His Tyr Lys Lys Lys Lys Asp	Ala Arg Phe Phe Ile Asn Asp Glu
1355	1360 1365
Ile Gly Ser Asn Asp Tyr Met	Tyr Asp Ile Lys Lys Lys Tyr Ser

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1370	1375	1380
Asn Asp Glu Asn Asn Tyr Lys 1385	Leu Asn Glu Lys Met Asn Ile Ser 1390	1395
Met Ser Asn Asp Glu Asp Met 1400	Ile Pro Thr Leu Asn Ser Glu His 1405	1410
Gly Asn Asn Phe Pro Ser Cys 1415	Gln Pro Asn Leu Leu Glu Lys Lys 1420	1425
Ser Thr Tyr Ile Asp Leu Asn 1430	Leu Tyr Asp Ser Asn Ser Met Asp 1435	1440
Asp Phe Thr Glu Glu Lys Tyr 1445	Asn Phe Val Asn Asn Glu Asn Asp 1450	1455
Leu Phe Asn Thr Lys Arg Trp 1460	Lys Phe Asn Phe Ser Lys Gly Lys 1465	1470
Asn Leu Phe Asn Asn Lys Phe 1475	Phe Asn Val Ser Asn Glu Asp Gly 1480	1485
Val Phe Ser Phe Phe Lys Asn 1490	Met Asn Leu Phe Arg Glu Leu Asn 1495	1500
Lys Ser Asn Asn Ser Leu Lys 1505	Leu Glu Ser Val Lys Asn Ser Asn 1510	1515
Asn Asn Cys Ser Asn Asn Lys 1520	Gly Asp Asp Asn Ile Gly Asn Met 1525	1530
Glu Asn Met Asn Thr Thr Asn 1535	Val Thr Ile Ala Ser Asp Glu His 1540	1545
Ile Ser Thr Lys Gly Asp Ile 1550	His Asp Glu Ser Phe Ser Arg Asp 1555	1560
Asp Asn Asp Cys Ile Leu Leu 1565	Lys Ile Glu Gly Arg Ser Lys Lys 1570	1575
Tyr Ser Asp Ile Thr Leu Tyr 1580	Asn Glu Asp Lys Ser Asn Leu Glu 1585	1590
Asn Asp Asn Glu Thr Ile Asn 1595	Glu Tyr Glu Asn Val Cys Ser Asn 1600	1605
Ile Asp Val Asn Glu Trp Glu 1610	Asp Lys Val Asn Gly Thr Cys Asn 1615	1620
Ser Val Gly Asp Lys Glu Thr 1625	Glu Lys Asn Asn Glu Lys Asn Asn 1630	1635
Glu Lys Asn Asn Glu Lys Asn 1640	Asn Glu Lys Asn Asn Glu Lys Asn 1645	1650
Asn Glu Lys Asn Asn Glu Lys 1655	Asn Asn Glu Lys Asn Asn Glu Glu 1660	1665
Asn Asn Glu Gly Asn Asn Glu 1670	Glu Asn Asn Glu Glu Asn Asn Glu 1675	1680
Glu Asn Asn Glu Glu Asn Asn 1685	Asp Ile Glu Lys Asn Asp Ile Lys 1690	1695
Asp Asn Asn Ser Gly Gln Val 1700	Lys Glu Asn Ile Ile Val Met Asn 1705	1710
Asn Thr Asn Asn Met Asp Val 1715	Asp Asn Asp Asp Asn Asn Asn Asn 1720	1725
Tyr Asn Asn Val Ser Thr Asp 1730	Glu Gly Ile Asp Ile Ile Lys Asn 1735	1740
Ile Lys Ser Glu Met Asn Asp 1745	Tyr Ile Tyr Asn Asp Asn Ile Met 1750	1755
Ile Lys Ile Asn Asn Lys Ser 1760	Ile Asp Leu Met Asn Ile Lys Asn 1765	1770

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Gln Lys	Asn Glu Pro Phe Leu	Asn Tyr Thr Asn Glu	Lys Asp Ile
1775	1780	1785	
His Met	Lys Ser Asn Ser Ser	Tyr Asn Val Asn Asp	Lys Met Asn
1790	1795	1800	
Leu Phe	Asn Asn Asn Glu Lys	Thr Glu Lys Asn Asn	Thr Ser Leu
1805	1810	1815	
Asn Asp	Leu Leu Tyr Lys Arg	Lys Glu Glu Leu Asp	Asp Glu Lys
1820	1825	1830	
Ile Ser	Glu Tyr Lys Asp Thr	Asn Leu Thr Asn Asn	Thr Phe Glu
1835	1840	1845	
His Ile	Ala Lys Arg Ile Asn	Leu Ile Leu Asn Asp	Thr Ile Glu
1850	1855	1860	
Phe Phe	Gln Lys His Thr Tyr	Leu His Asn Gly Tyr	Gly Asn Val
1865	1870	1875	
Gln Val	Cys Lys Lys Asn Lys	Arg Lys Leu Glu Lys	Lys Lys Leu
1880	1885	1890	
Lys Lys	Trp Ser Cys Ile Tyr	Lys Ile Asn Lys Ile	Val Arg Lys
1895	1900	1905	
Gly Ala	His Gly Val Val Phe	Ser Ala Trp Arg Ser	Glu Asn Val
1910	1915	1920	
Asp Phe	Phe Asn His Ser Phe	Phe Glu Asn Leu Asn	Leu Glu Asn
1925	1930	1935	
Lys Lys	Lys Gly Tyr Ile Asp	Glu Thr Asn Val Asn	Glu Asn Tyr
1940	1945	1950	
Glu Ser	Asp Asn Glu Tyr Asp	Ser Asp Glu Asp Asp	Thr Glu Ser
1955	1960	1965	
Asp Asn	Asp Asp Glu Gln Asn	Lys Glu Asn Glu Arg	Gly Asp Glu
1970	1975	1980	
Lys Asp	Gly Tyr Glu Glu Met	Asn Gly Gly Asp Lys	Asn Glu Glu
1985	1990	1995	
Met Asn	Gly Gly Asp Lys Asn	Glu Glu Met Asn Val	Gly Asp Lys
2000	2005	2010	
Asn Gly	Gly Ile Asn Glu Glu	His Lys Asn Glu Gly	Ile Asn Glu
2015	2020	2025	
Glu His	Lys Asp Glu Leu Ile	Asn Lys Glu His Lys	Asn Glu Arg
2030	2035	2040	
Ile Asn	Glu Glu His Lys Asn	Glu Arg Ile Asn Glu	Glu His Lys
2045	2050	2055	
Asn Glu	Gly Ile Asn Glu Glu	His Lys Asn Glu Gly	Ile Asn Glu
2060	2065	2070	
Glu His	Lys Asn Glu Arg Ile	Asn Glu Glu His Lys	Asn Glu Gly
2075	2080	2085	
Ile Asn	Lys Leu Thr Tyr His	Asn Met Asn Lys Asn	Asn Ile Ser
2090	2095	2100	
Asn Glu	Asn Asn Tyr Asn Asp	Asp Asp Ser Tyr Asp	Glu Asp Asn
2105	2110	2115	
Leu Val	Ser Leu Lys Ile Ile	Asn Leu Lys Tyr Leu	Ser Lys Lys
2120	2125	2130	
Asn Ser	Leu Lys Asn Ile Leu	Arg Glu Val Asn Phe	Leu Lys Met
2135	2140	2145	
Cys Glu	His Pro Asn Val Val	Lys Tyr Phe Glu Ser	Phe Phe Trp
2150	2155	2160	

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Pro	Pro	Cys	Tyr	Leu	Val	Ile	Val	Cys	Glu	Tyr	Leu	Ser	Gly	Gly
2165						2170					2175			
Thr	Leu	Tyr	Asp	Leu	Tyr	Lys	Asn	Tyr	Gly	Arg	Ile	Ser	Glu	Asp
2180						2185					2190			
Leu	Leu	Val	Tyr	Ile	Leu	Asp	Asp	Val	Leu	Asn	Gly	Leu	Asn	Tyr
2195						2200					2205			
Leu	His	Asn	Glu	Cys	Ser	Ser	Pro	Leu	Ile	His	Arg	Asp	Ile	Lys
2210						2215					2220			
Pro	Thr	Asn	Ile	Val	Leu	Ser	Lys	Asp	Gly	Ile	Ala	Lys	Ile	Ile
2225						2230					2235			
Asp	Phe	Gly	Ser	Cys	Glu	Glu	Leu	Lys	Asn	Ser	Asp	Gln	Ser	Lys
2240						2245					2250			
Glu	Leu	Val	Gly	Thr	Ile	Tyr	Tyr	Ile	Ser	Pro	Glu	Ile	Leu	Met
2255						2260					2265			
Arg	Thr	Asn	Tyr	Asp	Cys	Ser	Ser	Asp	Ile	Trp	Ser	Leu	Gly	Ile
2270						2275					2280			
Thr	Ile	Tyr	Glu	Ile	Val	Leu	Cys	Thr	Leu	Pro	Trp	Lys	Arg	Asn
2285						2290					2295			
Gln	Ser	Phe	Glu	Asn	Tyr	Ile	Lys	Thr	Ile	Ile	Asn	Ser	Ser	Pro
2300						2305					2310			
Lys	Ile	Asn	Ile	Thr	Glu	Gly	Tyr	Ser	Lys	His	Leu	Cys	Tyr	Phe
2315						2320					2325			
Val	Glu	Lys	Cys	Leu	Gln	Lys	Lys	Pro	Glu	Asn	Arg	Gly	Asn	Val
2330						2335					2340			
Lys	Asp	Leu	Leu	Asn	His	Lys	Phe	Leu	Ile	Lys	Lys	Arg	Tyr	Ile
2345						2350					2355			
Lys	Lys	Lys	Pro	Ser	Ser	Ile	Tyr	Glu	Ile	Arg	Asp	Ile	Leu	Lys
2360						2365					2370			
Ile	Tyr	Asn	Gly	Lys	Gly	Lys	Thr	Asn	Ile	Phe	Arg	Asn	Phe	Phe
2375						2380					2385			
Lys	Asn	Leu	Phe	Phe	Phe	Asn	Asp	Lys	Asn	Lys	Lys	Lys	Lys	Pro
2390						2395					2400			
Asn	Lys	Met	Ile	Ser	Ser	Lys	Ser	Cys	Asp	Ala	Glu	Met	Phe	Phe
2405						2410					2415			
Glu	Gln	Leu	Lys	Arg	Glu	Asn	Phe	Asp	Phe	Phe	Glu	Ile	Lys	Leu
2420						2425					2430			
Lys	Asp	Asp	Glu	Asn	Ser	Arg	Ser	Leu	Asn	Thr	Phe	Asn	Ile	Asn
2435						2440					2445			
Ile	Ser	Lys	Glu	Arg	Asp	Asp	Ile	Ser	Tyr	Ser	Ser	Leu	Asn	Leu
2450						2455					2460			
Glu	Lys	Ile	Lys	Glu	His	Ser	Leu	Asn	Met	Val	Ala	Ser	Val	Val
2465						2470					2475			
Gly	Thr	Glu	Gln	Ser	Gln	Lys								
2480						2485								

&lt;210&gt; SEQ ID NO 45

&lt;211&gt; LENGTH: 527

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Dictyostelium discoideum

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: SHK1 protein, kinase with gatekeeper Cys

&lt;400&gt; SEQUENCE: 45

Met Ala Thr Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln  
 1 5 10 15

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Ile	Lys	Ala	Arg	Lys	Asp	Ile	Gln	Ile	Gln	Gln	Ala	Gln	Ser	Ala	Ser
			20					25					30		
Asp	Ile	Leu	Gly	Pro	Pro	Glu	Ile	Ser	Glu	Thr	Glu	Ile	Thr	Thr	Glu
		35					40					45			
Ser	Ile	Leu	Gly	Asp	Gly	Ser	Phe	Gly	Thr	Val	Tyr	Lys	Gly	Arg	Cys
		50				55					60				
Lys	Leu	Lys	Asp	Val	Pro	Val	Lys	Val	Met	Leu	Lys	Gln	Val	Asp	Gln
		65			70					75					80
Lys	Thr	Leu	Thr	Asp	Phe	Arg	Lys	Glu	Val	Ala	Ile	Met	Ser	Lys	Ile
				85					90					95	
Phe	His	Pro	Asn	Ile	Val	Leu	Phe	Leu	Gly	Ala	Cys	Thr	Ser	Thr	Pro
			100					105					110		
Gly	Lys	Leu	Met	Ile	Cys	Thr	Glu	Leu	Met	Lys	Gly	Asn	Leu	Glu	Ser
		115					120					125			
Leu	Leu	Leu	Asp	Pro	Met	Val	Lys	Leu	Pro	Leu	Ile	Thr	Arg	Met	Arg
		130				135					140				
Met	Ala	Lys	Asp	Ala	Ala	Leu	Gly	Val	Leu	Trp	Leu	His	Ser	Ser	Asn
		145			150					155					160
Pro	Val	Phe	Ile	His	Arg	Asp	Leu	Lys	Thr	Ser	Asn	Leu	Leu	Val	Asp
				165					170					175	
Ala	Asn	Leu	Thr	Val	Lys	Val	Cys	Asp	Phe	Gly	Leu	Ser	Gln	Ile	Lys
			180					185					190		
Gln	Arg	Gly	Glu	Asn	Leu	Lys	Asp	Gly	Gln	Asp	Gly	Ala	Lys	Gly	Thr
		195					200					205			
Pro	Leu	Trp	Met	Ala	Pro	Glu	Val	Leu	Gln	Gly	Arg	Leu	Phe	Asn	Glu
		210				215					220				
Lys	Ala	Asp	Val	Tyr	Ser	Phe	Gly	Leu	Val	Leu	Trp	Gln	Ile	Phe	Thr
		225			230					235					240
Arg	Gln	Glu	Leu	Phe	Pro	Glu	Phe	Asp	Asn	Phe	Phe	Lys	Phe	Val	Ala
			245					250						255	
Ala	Ile	Cys	Glu	Lys	Gln	Leu	Arg	Pro	Ser	Ile	Pro	Asp	Asp	Cys	Pro
			260					265					270		
Lys	Ser	Leu	Lys	Glu	Leu	Ile	Gln	Lys	Cys	Trp	Asp	Pro	Asn	Pro	Glu
		275					280					285			
Val	Arg	Pro	Ser	Phe	Glu	Gly	Ile	Val	Ser	Glu	Leu	Glu	Glu	Ile	Ile
		290				295					300				
Ile	Asp	Cys	Cys	Ile	Pro	Asp	Glu	Tyr	Gly	Ala	Ile	Leu	Trp	Lys	Asn
		305			310					315					320
His	Phe	Lys	His	Glu	Asn	Glu	Ala	Asn	Trp	Lys	Asp	Phe	Ile	Asn	Val
			325						330					335	
Phe	Ser	Asn	Phe	Val	Gly	Leu	Thr	Asn	Ala	Asn	Thr	Pro	Ser	Met	Ser
			340					345					350		
Asp	Leu	Leu	Gln	Phe	Ser	Pro	Asn	Leu	Asn	Gly	Ser	Thr	Ile	Glu	Leu
		355					360					365			
Asn	Phe	Lys	Cys	Leu	Lys	Ser	Ile	Ile	Val	Ser	Ser	Pro	Lys	Gly	Pro
		370				375					380				
His	Glu	Glu	Glu	Val	Val	Leu	Met	Glu	Gln	Phe	Gly	Lys	Val	Leu	Ala
				390						395					400
Trp	Phe	Gly	Asn	Leu	Lys	Glu	Asp	Gly	Ser	Gln	Ile	Leu	Asp	Lys	Ile
			405					410					415		
Arg	Gln	Leu	Met	Glu	Cys	Ala	Trp	Phe	His	Gly	Asp	Ile	Ser	Thr	Ser
			420					425					430		
Glu	Ser	Glu	Asn	Arg	Leu	Arg	Gln	Lys	Pro	Glu	Gly	Thr	Phe	Leu	Val

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435	440	445
Arg Phe Ser Thr Ser Glu Tyr Gly Ala Tyr Thr Ile Ser Lys Val Ser		
450	455	460
Lys Asn Gly Gly Ile Ser His Gln Arg Ile His Arg Pro Gln Gly Lys		
465	470	475
Phe Gln Val Asn Asn Ser Lys Tyr Leu Ser Val Lys Glu Leu Ile Thr		
485	490	495
Gly Glu Ala Gln Ala Leu Gly Ile Asn Thr Pro Cys Leu Gly Ser Arg		
500	505	510
Phe Leu Phe Leu Ile Tyr Lys Ala Gln Leu Ser Gly Tyr Ile Asn		
515	520	525
 <210> SEQ ID NO 46		
<211> LENGTH: 523		
<212> TYPE: PRT		
<213> ORGANISM: Rous sarcoma virus		
<220> FEATURE:		
<223> OTHER INFORMATION: v-Src		
 <400> SEQUENCE: 46		
Met Gly Ser Ser Lys Ser Lys Pro Lys Asp Pro Ser Gln Arg Arg Arg		
1	5	10
Ser Leu Glu Pro Pro Asp Ser Thr His His Gly Gly Phe Pro Ala Ser		
20	25	30
Gln Thr Pro Asn Lys Thr Ala Ala Pro Asp Thr His Arg Thr Pro Ser		
35	40	45
Arg Ser Phe Gly Thr Val Ala Thr Glu Pro Lys Leu Phe Gly Asp Phe		
50	55	60
Asn Thr Ser Asp Thr Val Thr Ser Pro Gln Arg Ala Gly Ala Leu Ala		
65	70	75
Gly Gly Val Thr Thr Phe Val Ala Leu Tyr Asp Tyr Glu Ser Trp Ile		
85	90	95
Glu Thr Asp Leu Ser Phe Lys Lys Gly Glu Arg Leu Gln Ile Val Asn		
100	105	110
Asn Thr Glu Gly Asn Trp Trp Leu Ala His Ser Val Thr Thr Gly Gln		
115	120	125
Thr Gly Tyr Ile Pro Ser Asn Tyr Val Ala Pro Ser Asp Ser Ile Gln		
130	135	140
Ala Glu Glu Trp Tyr Phe Gly Lys Ile Thr Arg Arg Glu Ser Glu Arg		
145	150	155
Leu Leu Leu Asn Pro Glu Asn Pro Arg Gly Thr Phe Leu Val Arg Glu		
165	170	175
Ser Glu Thr Thr Lys Gly Ala Tyr Cys Leu Ser Val Ser Asp Phe Asp		
180	185	190
Asn Ala Lys Gly Leu Asn Val Lys His Tyr Lys Ile Arg Lys Leu Asp		
195	200	205
Ser Gly Gly Phe Tyr Ile Thr Ser Arg Thr Gln Phe Ser Ser Leu Gln		
210	215	220
Gln Leu Val Ala Tyr Tyr Ser Lys His Ala Asp Gly Leu Cys His Arg		
225	230	235
Leu Thr Asn Val Cys Pro Thr Ser Lys Pro Gln Thr Gln Gly Leu Ala		
245	250	255
Lys Asp Ala Trp Glu Ile Pro Arg Glu Ser Leu Arg Leu Glu Val Lys		
260	265	270
Leu Gly Gln Gly Cys Phe Gly Glu Val Trp Met Gly Thr Trp Asn Gly		

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275					280					285					
Thr	Thr	Arg	Val	Ala	Ile	Lys	Thr	Leu	Lys	Pro	Gly	Thr	Met	Ser	Pro
290						295					300				
Glu	Ala	Phe	Leu	Gln	Glu	Ala	Gln	Val	Met	Lys	Lys	Leu	Arg	His	Glu
305					310					315					320
Lys	Leu	Val	Gln	Leu	Tyr	Ala	Val	Val	Ser	Glu	Glu	Pro	Ile	Tyr	Ile
				325					330					335	
Val	Ile	Glu	Tyr	Met	Ser	Lys	Gly	Ser	Leu	Leu	Asp	Phe	Leu	Lys	Gly
			340					345					350		
Lys	Tyr	Leu	Arg	Leu	Pro	Gln	Leu	Val	Asp	Met	Ala	Ala	Gln	Ile	Ala
		355					360					365			
Ser	Gly	Met	Ala	Tyr	Val	Glu	Arg	Met	Asn	Tyr	Val	His	Arg	Asp	Leu
370						375					380				
Arg	Ala	Ala	Asn	Ile	Leu	Val	Gly	Glu	Asn	Leu	Val	Cys	Lys	Val	Ala
385					390					395					400
Asp	Phe	Gly	Leu	Ala	Arg	Leu	Ile	Glu	Asp	Asn	Glu	Tyr	Thr	Ala	Arg
				405					410					415	
Gln	Gly	Ala	Lys	Phe	Pro	Ile	Lys	Trp	Thr	Ala	Pro	Glu	Ala	Ala	Leu
			420					425					430		
Tyr	Gly	Arg	Phe	Thr	Ile	Lys	Ser	Asp	Val	Trp	Ser	Phe	Gly	Ile	Leu
		435					440					445			
Leu	Thr	Glu	Leu	Thr	Thr	Lys	Gly	Arg	Met	Pro	Tyr	Pro	Gly	Met	Gly
450						455					460				
Asn	Gly	Glu	Val	Leu	Asp	Arg	Val	Glu	Arg	Gly	Tyr	Arg	Met	Pro	Cys
465					470					475					480
Pro	Pro	Glu	Cys	Pro	Glu	Ser	Leu	His	Asp	Leu	Met	Cys	Gln	Cys	Trp
				485					490					495	
Arg	Arg	Asp	Pro	Glu	Glu	Arg	Pro	Thr	Phe	Glu	Tyr	Leu	Gln	Ala	Gln
			500					505					510		
Leu	Leu	Pro	Ala	Cys	Val	Leu	Glu	Val	Ala	Glu					
		515					520								
<210> SEQ ID NO 47															
<211> LENGTH: 523															
<212> TYPE: PRT															
<213> ORGANISM: Artificial Sequence															
<220> FEATURE:															
<223> OTHER INFORMATION: synthetic [I338X]v-Src															
<220> FEATURE:															
<221> NAME/KEY: VARIANT															
<222> LOCATION: (338)..(338)															
<223> OTHER INFORMATION: Xaa = any naturally occurring amino acid															
<400> SEQUENCE: 47															
Met	Gly	Ser	Ser	Lys	Ser	Lys	Pro	Lys	Asp	Pro	Ser	Gln	Arg	Arg	Arg
1				5					10				15		
Ser	Leu	Glu	Pro	Pro	Asp	Ser	Thr	His	His	Gly	Gly	Phe	Pro	Ala	Ser
			20					25					30		
Gln	Thr	Pro	Asn	Lys	Thr	Ala	Ala	Pro	Asp	Thr	His	Arg	Thr	Pro	Ser
		35					40					45			
Arg	Ser	Phe	Gly	Thr	Val	Ala	Thr	Glu	Pro	Lys	Leu	Phe	Gly	Asp	Phe
	50					55					60				
Asn	Thr	Ser	Asp	Thr	Val	Thr	Ser	Pro	Gln	Arg	Ala	Gly	Ala	Leu	Ala
65					70					75				80	
Gly	Gly	Val	Thr	Thr	Phe	Val	Ala	Leu	Tyr	Asp	Tyr	Glu	Ser	Trp	Ile
				85					90					95	

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Glu Thr Asp	Leu Ser Phe Lys Lys Gly	Glu Arg Leu Gln Ile Val Asn	
	100	105	110
Asn Thr Glu Gly Asn Trp Trp	Leu Ala His Ser Val Thr Thr Gly Gln		
	115	120	125
Thr Gly Tyr Ile Pro Ser Asn Tyr Val Ala Pro Ser Asp Ser Ile Gln			
	130	135	140
Ala Glu Glu Trp Tyr Phe Gly Lys Ile Thr Arg Arg Glu Ser Glu Arg			
	145	150	155
Leu Leu Leu Asn Pro Glu Asn Pro Arg Gly Thr Phe Leu Val Arg Glu			
	165	170	175
Ser Glu Thr Thr Lys Gly Ala Tyr Cys Leu Ser Val Ser Asp Phe Asp			
	180	185	190
Asn Ala Lys Gly Leu Asn Val Lys His Tyr Lys Ile Arg Lys Leu Asp			
	195	200	205
Ser Gly Gly Phe Tyr Ile Thr Ser Arg Thr Gln Phe Ser Ser Leu Gln			
	210	215	220
Gln Leu Val Ala Tyr Tyr Ser Lys His Ala Asp Gly Leu Cys His Arg			
	225	230	235
Leu Thr Asn Val Cys Pro Thr Ser Lys Pro Gln Thr Gln Gly Leu Ala			
	245	250	255
Lys Asp Ala Trp Glu Ile Pro Arg Glu Ser Leu Arg Leu Glu Val Lys			
	260	265	270
Leu Gly Gln Gly Cys Phe Gly Glu Val Trp Met Gly Thr Trp Asn Gly			
	275	280	285
Thr Thr Arg Val Ala Ile Lys Thr Leu Lys Pro Gly Thr Met Ser Pro			
	290	295	300
Glu Ala Phe Leu Gln Glu Ala Gln Val Met Lys Lys Leu Arg His Glu			
	305	310	315
Lys Leu Val Gln Leu Tyr Ala Val Val Ser Glu Glu Pro Ile Tyr Ile			
	325	330	335
Val Xaa Glu Tyr Met Ser Lys Gly Ser Leu Leu Asp Phe Leu Lys Gly			
	340	345	350
Lys Tyr Leu Arg Leu Pro Gln Leu Val Asp Met Ala Ala Gln Ile Ala			
	355	360	365
Ser Gly Met Ala Tyr Val Glu Arg Met Asn Tyr Val His Arg Asp Leu			
	370	375	380
Arg Ala Ala Asn Ile Leu Val Gly Glu Asn Leu Val Cys Lys Val Ala			
	385	390	395
Asp Phe Gly Leu Ala Arg Leu Ile Glu Asp Asn Glu Tyr Thr Ala Arg			
	405	410	415
Gln Gly Ala Lys Phe Pro Ile Lys Trp Thr Ala Pro Glu Ala Ala Leu			
	420	425	430
Tyr Gly Arg Phe Thr Ile Lys Ser Asp Val Trp Ser Phe Gly Ile Leu			
	435	440	445
Leu Thr Glu Leu Thr Thr Lys Gly Arg Met Pro Tyr Pro Gly Met Gly			
	450	455	460
Asn Gly Glu Val Leu Asp Arg Val Glu Arg Gly Tyr Arg Met Pro Cys			
	465	470	475
Pro Pro Glu Cys Pro Glu Ser Leu His Asp Leu Met Cys Gln Cys Trp			
	485	490	495
Arg Arg Asp Pro Glu Glu Arg Pro Thr Phe Glu Tyr Leu Gln Ala Gln			
	500	505	510
Leu Leu Pro Ala Cys Val Leu Glu Val Ala Glu			

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515  
 520

<210> SEQ ID NO 48  
 <211> LENGTH: 523  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: synthetic [I338C]v-Src

<400> SEQUENCE: 48

Met Gly Ser Ser Lys Ser Lys Pro Lys Asp Pro Ser Gln Arg Arg Arg  
 1 5 10 15  
 Ser Leu Glu Pro Pro Asp Ser Thr His His Gly Gly Phe Pro Ala Ser  
 20 25 30  
 Gln Thr Pro Asn Lys Thr Ala Ala Pro Asp Thr His Arg Thr Pro Ser  
 35 40 45  
 Arg Ser Phe Gly Thr Val Ala Thr Glu Pro Lys Leu Phe Gly Asp Phe  
 50 55 60  
 Asn Thr Ser Asp Thr Val Thr Ser Pro Gln Arg Ala Gly Ala Leu Ala  
 65 70 75 80  
 Gly Gly Val Thr Thr Phe Val Ala Leu Tyr Asp Tyr Glu Ser Trp Ile  
 85 90 95  
 Glu Thr Asp Leu Ser Phe Lys Lys Gly Glu Arg Leu Gln Ile Val Asn  
 100 105 110  
 Asn Thr Glu Gly Asn Trp Trp Leu Ala His Ser Val Thr Thr Gly Gln  
 115 120 125  
 Thr Gly Tyr Ile Pro Ser Asn Tyr Val Ala Pro Ser Asp Ser Ile Gln  
 130 135 140  
 Ala Glu Glu Trp Tyr Phe Gly Lys Ile Thr Arg Arg Glu Ser Glu Arg  
 145 150 155 160  
 Leu Leu Leu Asn Pro Glu Asn Pro Arg Gly Thr Phe Leu Val Arg Glu  
 165 170 175  
 Ser Glu Thr Thr Lys Gly Ala Tyr Cys Leu Ser Val Ser Asp Phe Asp  
 180 185 190  
 Asn Ala Lys Gly Leu Asn Val Lys His Tyr Lys Ile Arg Lys Leu Asp  
 195 200 205  
 Ser Gly Gly Phe Tyr Ile Thr Ser Arg Thr Gln Phe Ser Ser Leu Gln  
 210 215 220  
 Gln Leu Val Ala Tyr Tyr Ser Lys His Ala Asp Gly Leu Cys His Arg  
 225 230 235 240  
 Leu Thr Asn Val Cys Pro Thr Ser Lys Pro Gln Thr Gln Gly Leu Ala  
 245 250 255  
 Lys Asp Ala Trp Glu Ile Pro Arg Glu Ser Leu Arg Leu Glu Val Lys  
 260 265 270  
 Leu Gly Gln Gly Cys Phe Gly Glu Val Trp Met Gly Thr Trp Asn Gly  
 275 280 285  
 Thr Thr Arg Val Ala Ile Lys Thr Leu Lys Pro Gly Thr Met Ser Pro  
 290 295 300  
 Glu Ala Phe Leu Gln Glu Ala Gln Val Met Lys Lys Leu Arg His Glu  
 305 310 315 320  
 Lys Leu Val Gln Leu Tyr Ala Val Val Ser Glu Glu Pro Ile Tyr Ile  
 325 330 335  
 Val Cys Glu Tyr Met Ser Lys Gly Ser Leu Leu Asp Phe Leu Lys Gly  
 340 345 350  
 Lys Tyr Leu Arg Leu Pro Gln Leu Val Asp Met Ala Ala Gln Ile Ala

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355	360	365
Ser Gly Met Ala Tyr Val	Glu Arg Met Asn Tyr Val	His Arg Asp Leu
370	375	380
Arg Ala Ala Asn Ile Leu Val	Gly Glu Asn Leu Val	Cys Lys Val Ala
385	390	395 400
Asp Phe Gly Leu Ala Arg Leu	Ile Glu Asp Asn Glu Tyr Thr	Ala Arg
	405	410 415
Gln Gly Ala Lys Phe Pro Ile	Lys Trp Thr Ala Pro Glu Ala	Ala Leu
	420	425 430
Tyr Gly Arg Phe Thr Ile Lys	Ser Asp Val Trp Ser Phe Gly	Ile Leu
	435	440 445
Leu Thr Glu Leu Thr Thr Lys	Gly Arg Met Pro Tyr Pro	Gly Met Gly
	450	455 460
Asn Gly Glu Val Leu Asp Arg	Val Glu Arg Gly Tyr Arg	Met Pro Cys
465	470	475 480
Pro Pro Glu Cys Pro Glu Ser	Leu His Asp Leu Met Cys	Gln Cys Trp
	485	490 495
Arg Arg Asp Pro Glu Glu Arg	Pro Thr Phe Glu Tyr Leu	Gln Ala Gln
	500	505 510
Leu Leu Pro Ala Cys Val Leu	Glu Val Ala Glu	
	515	520

&lt;210&gt; SEQ ID NO 49

&lt;211&gt; LENGTH: 523

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: synthetic [I338T]v-Src

&lt;400&gt; SEQUENCE: 49

Met Gly Ser Ser Lys Ser Lys	Pro Lys Asp Pro Ser Gln	Arg Arg Arg
1	5	10 15
Ser Leu Glu Pro Pro Asp Ser	Thr His His Gly Gly Phe	Pro Ala Ser
	20	25 30
Gln Thr Pro Asn Lys Thr Ala	Ala Pro Asp Thr His Arg	Thr Pro Ser
	35	40 45
Arg Ser Phe Gly Thr Val Ala	Thr Glu Pro Lys Leu Phe	Gly Asp Phe
	50	55 60
Asn Thr Ser Asp Thr Val Thr	Ser Pro Gln Arg Ala Gly	Ala Leu Ala
	65	70 75 80
Gly Gly Val Thr Thr Phe Val	Ala Leu Tyr Asp Tyr Glu	Ser Trp Ile
	85	90 95
Glu Thr Asp Leu Ser Phe Lys	Lys Gly Glu Arg Leu Gln	Ile Val Asn
	100	105 110
Asn Thr Glu Gly Asn Trp Trp	Leu Ala His Ser Val Thr	Thr Gly Gln
	115	120 125
Thr Gly Tyr Ile Pro Ser Asn	Tyr Val Ala Pro Ser Asp	Ser Ile Gln
	130	135 140
Ala Glu Glu Trp Tyr Phe Gly	Lys Ile Thr Arg Arg Glu	Ser Glu Arg
	145	150 155 160
Leu Leu Leu Asn Pro Glu Asn	Pro Arg Gly Thr Phe Leu	Val Arg Glu
	165	170 175
Ser Glu Thr Thr Lys Gly Ala	Tyr Cys Leu Ser Val Ser	Asp Phe Asp
	180	185 190
Asn Ala Lys Gly Leu Asn Val	Lys His Tyr Lys Ile Arg	Lys Leu Asp

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195	200	205
Ser Gly Gly Phe Tyr Ile Thr Ser Arg Thr Gln Phe Ser Ser Leu Gln 210 215 220		
Gln Leu Val Ala Tyr Tyr Ser Lys His Ala Asp Gly Leu Cys His Arg 225 230 235 240		
Leu Thr Asn Val Cys Pro Thr Ser Lys Pro Gln Thr Gln Gly Leu Ala 245 250 255		
Lys Asp Ala Trp Glu Ile Pro Arg Glu Ser Leu Arg Leu Glu Val Lys 260 265 270		
Leu Gly Gln Gly Cys Phe Gly Glu Val Trp Met Gly Thr Trp Asn Gly 275 280 285		
Thr Thr Arg Val Ala Ile Lys Thr Leu Lys Pro Gly Thr Met Ser Pro 290 295 300		
Glu Ala Phe Leu Gln Glu Ala Gln Val Met Lys Lys Leu Arg His Glu 305 310 315 320		
Lys Leu Val Gln Leu Tyr Ala Val Val Ser Glu Glu Pro Ile Tyr Ile 325 330 335		
Val Thr Glu Tyr Met Ser Lys Gly Ser Leu Leu Asp Phe Leu Lys Gly 340 345 350		
Lys Tyr Leu Arg Leu Pro Gln Leu Val Asp Met Ala Ala Gln Ile Ala 355 360 365		
Ser Gly Met Ala Tyr Val Glu Arg Met Asn Tyr Val His Arg Asp Leu 370 375 380		
Arg Ala Ala Asn Ile Leu Val Gly Glu Asn Leu Val Cys Lys Val Ala 385 390 395 400		
Asp Phe Gly Leu Ala Arg Leu Ile Glu Asp Asn Glu Tyr Thr Ala Arg 405 410 415		
Gln Gly Ala Lys Phe Pro Ile Lys Trp Thr Ala Pro Glu Ala Ala Leu 420 425 430		
Tyr Gly Arg Phe Thr Ile Lys Ser Asp Val Trp Ser Phe Gly Ile Leu 435 440 445		
Leu Thr Glu Leu Thr Thr Lys Gly Arg Met Pro Tyr Pro Gly Met Gly 450 455 460		
Asn Gly Glu Val Leu Asp Arg Val Glu Arg Gly Tyr Arg Met Pro Cys 465 470 475 480		
Pro Pro Glu Cys Pro Glu Ser Leu His Asp Leu Met Cys Gln Cys Trp 485 490 495		
Arg Arg Asp Pro Glu Glu Arg Pro Thr Phe Glu Tyr Leu Gln Ala Gln 500 505 510		
Leu Leu Pro Ala Cys Val Leu Glu Val Ala Glu 515 520		

&lt;210&gt; SEQ ID NO 50

&lt;211&gt; LENGTH: 523

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: synthetic [I338G]v-Src

&lt;400&gt; SEQUENCE: 50

Met Gly Ser Ser Lys Ser Lys Pro Lys Asp Pro Ser Gln Arg Arg Arg  
1 5 10 15

Ser Leu Glu Pro Pro Asp Ser Thr His His Gly Gly Phe Pro Ala Ser  
20 25 30

Gln Thr Pro Asn Lys Thr Ala Ala Pro Asp Thr His Arg Thr Pro Ser

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35	40	45
Arg Ser Phe Gly Thr Val	Ala Thr Glu Pro Lys	Leu Phe Gly Asp Phe
50	55	60
Asn Thr Ser Asp Thr Val	Thr Ser Pro Gln Arg	Ala Gly Ala Leu Ala
65	70	75
Gly Gly Val Thr Thr Phe	Val Ala Leu Tyr Asp	Tyr Glu Ser Trp Ile
85	90	95
Glu Thr Asp Leu Ser Phe	Lys Lys Gly Glu Arg	Leu Gln Ile Val Asn
100	105	110
Asn Thr Glu Gly Asn Trp	Trp Leu Ala His Ser	Val Thr Thr Gly Gln
115	120	125
Thr Gly Tyr Ile Pro Ser	Asn Tyr Val Ala Pro	Ser Asp Ser Ile Gln
130	135	140
Ala Glu Glu Trp Tyr Phe	Gly Lys Ile Thr Arg	Arg Glu Ser Glu Arg
145	150	155
Leu Leu Leu Asn Pro Glu	Asn Pro Arg Gly Thr	Phe Leu Val Arg Glu
165	170	175
Ser Glu Thr Thr Lys Gly	Ala Tyr Cys Leu Ser	Val Ser Asp Phe Asp
180	185	190
Asn Ala Lys Gly Leu Asn	Val Lys His Tyr Lys	Ile Arg Lys Leu Asp
195	200	205
Ser Gly Gly Phe Tyr Ile	Thr Ser Arg Thr Gln	Phe Ser Ser Leu Gln
210	215	220
Gln Leu Val Ala Tyr Tyr	Ser Lys His Ala Asp	Gly Leu Cys His Arg
225	230	235
Leu Thr Asn Val Cys Pro	Thr Ser Lys Pro Gln	Thr Gln Gly Leu Ala
245	250	255
Lys Asp Ala Trp Glu Ile	Pro Arg Glu Ser Leu	Arg Leu Glu Val Lys
260	265	270
Leu Gly Gln Gly Cys Phe	Gly Glu Val Trp Met	Gly Thr Trp Asn Gly
275	280	285
Thr Thr Arg Val Ala Ile	Lys Thr Leu Lys Pro	Gly Thr Met Ser Pro
290	295	300
Glu Ala Phe Leu Gln Glu	Ala Gln Val Met Lys	Lys Leu Arg His Glu
305	310	315
Lys Leu Val Gln Leu Tyr	Ala Val Val Ser Glu	Glu Pro Ile Tyr Ile
325	330	335
Val Gly Glu Tyr Met Ser	Lys Gly Ser Leu Leu	Asp Phe Leu Lys Gly
340	345	350
Lys Tyr Leu Arg Leu Pro	Gln Leu Val Asp Met	Ala Ala Gln Ile Ala
355	360	365
Ser Gly Met Ala Tyr Val	Glu Arg Met Asn Tyr	Val His Arg Asp Leu
370	375	380
Arg Ala Ala Asn Ile Leu	Val Gly Glu Asn Leu	Val Cys Lys Val Ala
385	390	395
Asp Phe Gly Leu Ala Arg	Leu Ile Glu Asp Asn	Glu Tyr Thr Ala Arg
405	410	415
Gln Gly Ala Lys Phe Pro	Ile Lys Trp Thr Ala	Pro Glu Ala Ala Leu
420	425	430
Tyr Gly Arg Phe Thr Ile	Lys Ser Asp Val Trp	Ser Phe Gly Ile Leu
435	440	445
Leu Thr Glu Leu Thr Thr	Lys Gly Arg Met Pro	Tyr Pro Gly Met Gly
450	455	460

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Asn Gly Glu Val Leu Asp Arg Val Glu Arg Gly Tyr Arg Met Pro Cys  
465 470 475 480

Pro Pro Glu Cys Pro Glu Ser Leu His Asp Leu Met Cys Gln Cys Trp  
485 490 495

Arg Arg Asp Pro Glu Glu Arg Pro Thr Phe Glu Tyr Leu Gln Ala Gln  
500 505 510

Leu Leu Pro Ala Cys Val Leu Glu Val Ala Glu  
515 520

<210> SEQ ID NO 51  
<211> LENGTH: 8  
<212> TYPE: PRT  
<213> ORGANISM: Artificial sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: synthetic kinase peptide substrate

<400> SEQUENCE: 51

Ile Tyr Gly Glu Phe Lys Lys Lys  
1 5

<210> SEQ ID NO 52  
<211> LENGTH: 38  
<212> TYPE: PRT  
<213> ORGANISM: Artificial sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: synthetic ATP binding site region of src kinase domain

<400> SEQUENCE: 52

Pro Glu Ala Phe Leu Gln Glu Ala Gln Val Met Lys Lys Leu Arg His  
1 5 10 15

Glu Lys Leu Val Gln Leu Tyr Ala Val Val Ser Glu Glu Pro Ile Tyr  
20 25 30

Ile Val Thr Glu Tyr Met  
35

<210> SEQ ID NO 53  
<211> LENGTH: 40  
<212> TYPE: PRT  
<213> ORGANISM: Artificial sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: synthetic ATP binding site region of rsk2 kinase domain

<400> SEQUENCE: 53

Lys Arg Asp Pro Thr Glu Glu Ile Glu Ile Leu Leu Arg Tyr Gly Gln  
1 5 10 15

His Pro Asn Ile Ile Thr Leu Lys Asp Val Tyr Asp Asp Gly Lys Tyr  
20 25 30

Val Tyr Val Val Thr Glu Leu Met  
35 40

<210> SEQ ID NO 54  
<211> LENGTH: 44  
<212> TYPE: PRT  
<213> ORGANISM: Artificial sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: synthetic ATP binding site region of nek2 kinase domain

<400> SEQUENCE: 54

Glu Val Glu Lys Gln Met Leu Val Ser Glu Val Asn Leu Leu Arg Glu  
1 5 10 15

-continued

Leu Lys His Pro Asn Ile Val Arg Tyr Tyr Asp Arg Ile Ile Asp Arg  
                   20                  25                  30

Thr Asn Thr Thr Leu Tyr Ile Val Met Glu Tyr Cys  
           35                  40

<210> SEQ ID NO 55  
 <211> LENGTH: 43  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: synthetic ATP binding site region of mekk1  
                   kinase domain

<400> SEQUENCE: 55

Gln Glu Glu Val Val Glu Ala Leu Arg Glu Glu Ile Arg Met Met Ser  
 1                  5                  10                  15

His Leu Asn His Pro Asn Ile Ile Arg Met Leu Gly Ala Thr Cys Glu  
           20                  25                  30

Lys Ser Asn Tyr Asn Leu Phe Ile Glu Trp Met  
           35                  40

<210> SEQ ID NO 56  
 <211> LENGTH: 41  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: synthetic ATP binding site region of msk1  
                   kinase domain

<400> SEQUENCE: 56

Met Glu Ala Asn Thr Gln Lys Glu Ile Thr Ala Leu Lys Leu Cys Glu  
 1                  5                  10                  15

Gly His Pro Asn Ile Val Lys Leu His Glu Val Phe His Asp Gln Leu  
           20                  25                  30

His Thr Phe Leu Val Met Glu Leu Leu  
           35                  40

<210> SEQ ID NO 57  
 <211> LENGTH: 42  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: synthetic ATP binding site region of plk1  
                   kinase domain

<400> SEQUENCE: 57

Pro His Gln Arg Glu Lys Met Ser Met Glu Ile Ser Ile His Arg Ser  
 1                  5                  10                  15

Leu Ala His Gln His Val Val Gly Phe His Gly Phe Phe Glu Asp Asn  
           20                  25                  30

Asp Phe Val Phe Val Val Leu Glu Leu Cys  
           35                  40

<210> SEQ ID NO 58  
 <211> LENGTH: 40  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: synthetic region of v-Src kinase domain

<400> SEQUENCE: 58

Arg His Glu Lys Leu Val Gln Leu Tyr Ala Met Val Ser Glu Glu Pro  
 1                  5                  10                  15

Ile Tyr Ile Val Thr Glu Phe Met Ser Lys Gly Ser Leu Leu Asp Phe  
20 25 30

-continued

Leu Lys Glu Gly Asp Gly Lys Tyr  
           35                  40

<210> SEQ ID NO 63  
 <211> LENGTH: 40  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: synthetic region of Yrk kinase domain

<400> SEQUENCE: 63

Arg His Asp Lys Leu Val Gln Leu Tyr Ala Val Val Ser Glu Glu Pro  
 1          5                  10                  15

Ile Tyr Ile Val Thr Glu Phe Met Ser Gln Gly Ser Leu Leu Asp Phe  
           20                  25                  30

Leu Lys Asp Gly Asp Gly Arg Tyr  
           35                  40

<210> SEQ ID NO 64  
 <211> LENGTH: 40  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: synthetic region of c-Fgr kinase domain

<400> SEQUENCE: 64

Arg His Asp Lys Leu Val Gln Leu Tyr Ala Val Val Ser Glu Glu Pro  
 1          5                  10                  15

Ile Tyr Ile Val Thr Glu Phe Met Cys His Gly Ser Leu Leu Asp Phe  
           20                  25                  30

Leu Lys Asn Pro Glu Gly Gln Asp  
           35                  40

<210> SEQ ID NO 65  
 <211> LENGTH: 41  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: synthetic region of Lyn kinase domain

<400> SEQUENCE: 65

Gln His Asp Lys Leu Val Arg Leu Tyr Ala Val Val Thr Arg Glu Glu  
 1          5                  10                  15

Pro Ile Tyr Ile Ile Thr Glu Tyr Met Ala Lys Gly Ser Leu Leu Asp  
           20                  25                  30

Phe Leu Lys Ser Asp Glu Gly Gly Lys  
           35                  40

<210> SEQ ID NO 66  
 <211> LENGTH: 40  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: synthetic region of Hck kinase domain

<400> SEQUENCE: 66

Gln His Asp Lys Leu Val Lys Leu His Ala Val Val Thr Lys Glu Pro  
 1          5                  10                  15

Ile Tyr Ile Ile Thr Glu Phe Met Ala Lys Gly Ser Leu Leu Asp Phe  
           20                  25                  30

Leu Lys Ser Asp Glu Gly Ser Lys  
           35                  40

-continued

<210> SEQ ID NO 67  
 <211> LENGTH: 40  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: synthetic region of Blk kinase domain

<400> SEQUENCE: 67

Gln His Glu Arg Leu Val Arg Leu Tyr Ala Val Val Thr Arg Glu Pro  
 1 5 10 15  
 Ile Tyr Ile Val Thr Glu Tyr Met Ala Arg Gly Cys Leu Leu Asp Phe  
 20 25 30  
 Leu Lys Thr Asp Glu Gly Ser Arg  
 35 40

<210> SEQ ID NO 68  
 <211> LENGTH: 41  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: synthetic region of Abl kinase domain

<400> SEQUENCE: 68

Lys His Pro Asn Leu Val Gln Leu Leu Gly Val Cys Thr Arg Glu Pro  
 1 5 10 15  
 Pro Phe Tyr Ile Ile Thr Glu Phe Met Thr Tyr Gly Asn Leu Leu Asp  
 20 25 30  
 Tyr Leu Arg Glu Cys Asn Arg Gln Glu  
 35 40

<210> SEQ ID NO 69  
 <211> LENGTH: 40  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: synthetic region of Btk kinase domain

<400> SEQUENCE: 69

Ser His Glu Lys Leu Val Gln Leu Tyr Gly Val Cys Thr Lys Gln Arg  
 1 5 10 15  
 Pro Ile Phe Ile Ile Thr Glu Tyr Met Ala Asn Gly Cys Leu Leu Asn  
 20 25 30  
 Tyr Leu Arg Glu Met Arg His Arg  
 35 40

<210> SEQ ID NO 70  
 <211> LENGTH: 42  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: synthetic region of Csk kinase domain

<400> SEQUENCE: 70

Arg His Ser Asn Leu Val Gln Leu Leu Gly Val Ile Val Glu Glu Lys  
 1 5 10 15  
 Gly Gly Leu Tyr Ile Val Thr Glu Tyr Met Ala Lys Gly Ser Leu Val  
 20 25 30  
 Asp Tyr Leu Arg Ser Arg Gly Arg Ser Val  
 35 40

<210> SEQ ID NO 71

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<211> LENGTH: 41  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: synthetic region of PDGFR kinase domain

<400> SEQUENCE: 71

Pro His Leu Asn Val Val Asn Leu Leu Gly Ala Cys Thr Lys Gly Gly  
 1 5 10 15

Pro Ile Tyr Ile Ile Thr Glu Tyr Cys Arg Tyr Gly Asp Leu Val Asp  
 20 25 30

Tyr Leu His Arg Asn Lys His Thr Phe  
 35 40

<210> SEQ ID NO 72  
 <211> LENGTH: 41  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: synthetic region of p38 kinase domain

<400> SEQUENCE: 72

Gly Leu Leu Asp Val Phe Thr Pro Ala Arg Ser Leu Glu Glu Phe Asn  
 1 5 10 15

Asp Val Val Leu Val Thr His Leu Met Gly Ala Asp Leu Asn Asn Ile  
 20 25 30

Val Lys Cys Gln Lys Leu Thr Asp Asp  
 35 40

<210> SEQ ID NO 73  
 <211> LENGTH: 39  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: synthetic region of ZAP-70 kinase domain

<400> SEQUENCE: 73

Asp Asn Pro Tyr Ile Val Arg Leu Ile Gly Val Cys Gln Ala Glu Ala  
 1 5 10 15

Leu Met Leu Val Met Glu Met Ala Gly Gly Gly Pro Leu His Lys Phe  
 20 25 30

Leu Val Gly Lys Arg Glu Glu  
 35

<210> SEQ ID NO 74  
 <211> LENGTH: 42  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: synthetic region of JAK2 kinase domain

<400> SEQUENCE: 74

Gln His Asp Asn Ile Val Lys Tyr Lys Gly Val Cys Tyr Ser Ala Gly  
 1 5 10 15

Arg Arg Asn Leu Arg Leu Ile Met Glu Tyr Leu Pro Tyr Gly Ser Leu  
 20 25 30

Arg Asp Tyr Leu Gln Lys His Lys Glu Arg  
 35 40

<210> SEQ ID NO 75  
 <211> LENGTH: 39  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial sequence

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<220> FEATURE:
<223> OTHER INFORMATION: synthetic region of PKA kinase domain

<400> SEQUENCE: 75

Asn Phe Pro Phe Leu Val Lys Leu Glu Phe Ser Phe Lys Asp Asn Ser
1             5             10             15

Asn Leu Tyr Met Val Met Glu Tyr Val Pro Gly Gly Glu Met Phe Ser
20             25             30

His Leu Arg Arg Ile Gly Arg
35

<210> SEQ ID NO 76
<211> LENGTH: 39
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic region of CamK II kinase domain

<400> SEQUENCE: 76

Lys His Pro Asn Ile Val Arg Leu His Asp Ser Ile Ser Glu Glu Gly
1             5             10             15

His His Tyr Leu Ile Phe Asp Leu Val Thr Gly Gly Glu Leu Phe Glu
20             25             30

Asp Ile Val Ala Arg Glu Tyr
35

<210> SEQ ID NO 77
<211> LENGTH: 40
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic region of Cdk2 kinase domain

<400> SEQUENCE: 77

Asn His Pro Asn Ile Val Lys Leu Leu Asp Val Ile His Thr Glu Asn
1             5             10             15

Lys Leu Tyr Leu Val Phe Glu Phe Leu His Gln Asp Leu Lys Lys Phe
20             25             30

Met Asp Ala Ser Ala Leu Thr Gly
35             40

<210> SEQ ID NO 78
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic N-terminal His-6 tag

<400> SEQUENCE: 78

His His His His His His
1             5

<210> SEQ ID NO 79
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: synthetic spacer sequence

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&lt;400&gt; SEQUENCE: 79

Asp Tyr Asp Ile Pro Thr Thr  
1 5

&lt;210&gt; SEQ ID NO 80

&lt;211&gt; LENGTH: 7

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial sequence

&lt;220&gt; FEATURE:

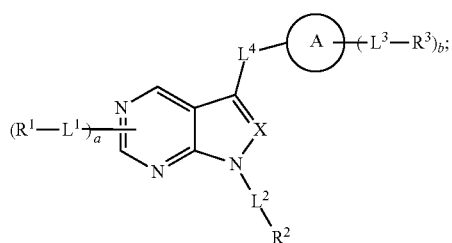
&lt;223&gt; OTHER INFORMATION: synthetic tobacco etch virus (TEV) protease site

&lt;400&gt; SEQUENCE: 80

Glu Asn Leu Tyr Phe Gln Gly  
1 5

What is claimed is:

1. A compound having the formula:



wherein:

X is =N—;

Ring A is phenyl;

L<sup>1</sup> is a bond;L<sup>2</sup> is a bond;L<sup>3</sup> is —C(O)—, —S(O)<sub>2</sub>—, or —NHS(O)<sub>2</sub>—;L<sup>4</sup> is unsubstituted C<sub>1</sub>-C<sub>5</sub> alkylene;R<sup>1</sup> is hydrogen or —NH<sub>2</sub>;R<sup>2</sup> is substituted or unsubstituted alkyl or substituted or unsubstituted cycloalkyl;R<sup>3</sup> is substituted or unsubstituted alkyl;

a is 1;

b is 1;

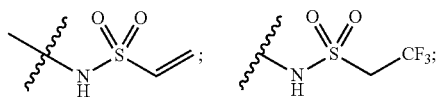
and

the salts and isomers thereof.

2. The compound of claim 1, wherein

L<sup>3</sup> is —C(O)—, —S(O)<sub>2</sub>—, or —NHS(O)<sub>2</sub>—; andR<sup>3</sup> is unsubstituted alkyl or alkyl substituted with chloro, fluoro, methyl, difluoromethyl, or trifluoromethyl.

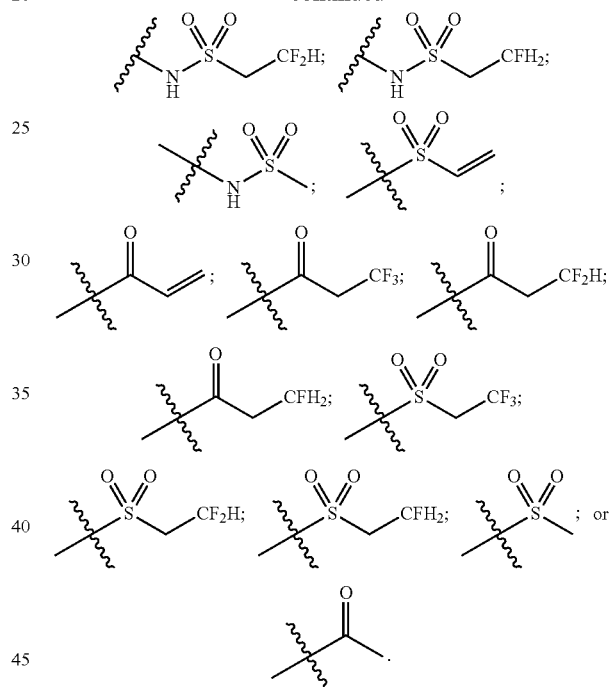
3. The compound of claim 2, wherein R<sup>3</sup> is ethenyl, ethyl, 2,2,2-trichloroethyl, 2,2-dichloroethyl, 2-chloroethyl, 2,2,2-trifluoroethyl, 2,2-difluoroethyl, or 2-fluoroethyl, propyl, isopropyl, 1-propenyl, or 2-propenyl.

4. The compound of claim 3, wherein —L<sup>3</sup>-R<sup>3</sup> is:

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(I)



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5. The compound of claim 1, wherein,

L<sup>1</sup> is a bond; andR<sup>1</sup> is hydrogen.

55

6. The compound of claim 1, wherein

L<sup>1</sup> is a bond; andR<sup>1</sup> is NH<sub>2</sub>.

60

7. The compound of claim 1, wherein

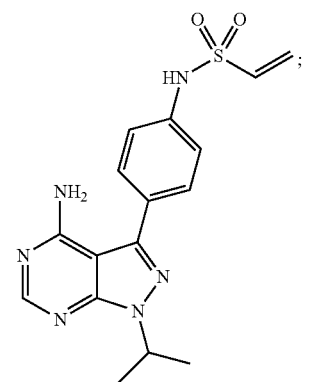
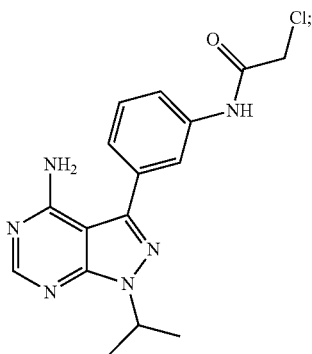
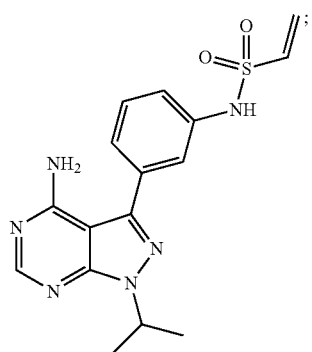
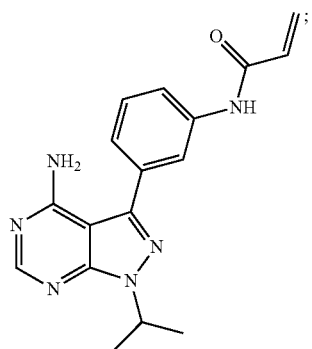
L<sup>2</sup> is a bond; andR<sup>2</sup> is methyl, ethyl, propyl, isopropyl, butyl, tert-butyl, pentyl, cyclopentyl, hexyl, or cyclohexyl.

65

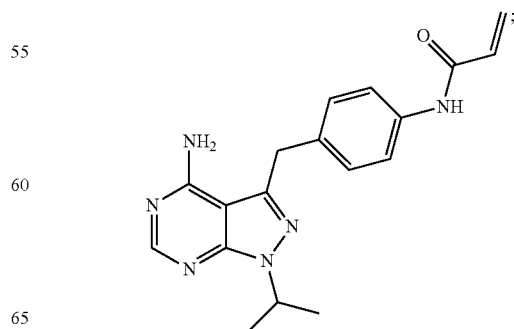
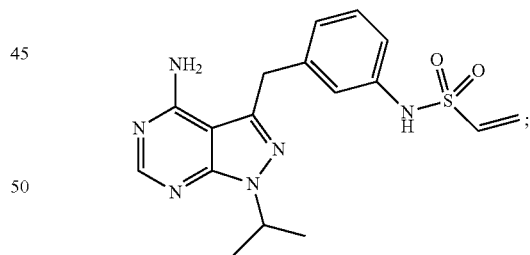
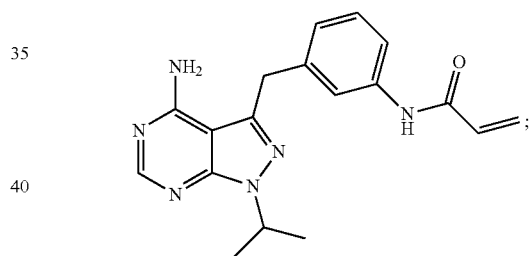
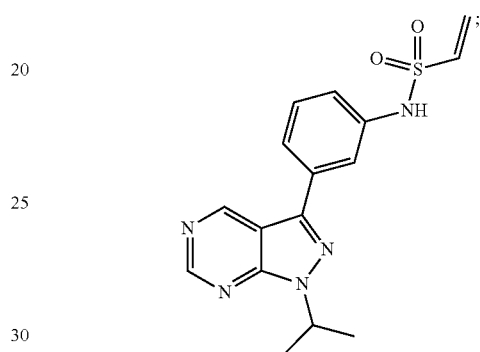
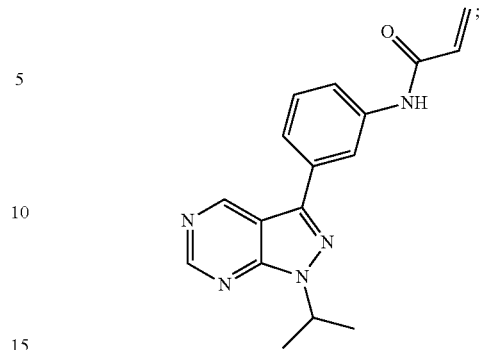
8. The compound of claim 7, wherein R<sup>2</sup> is isopropyl or cyclopentyl.9. The compound of claim 8, wherein R<sup>2</sup> is isopropyl.10. The compound of claim 8, wherein R<sup>2</sup> is cyclopentyl.

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11. A compound, having the formula:

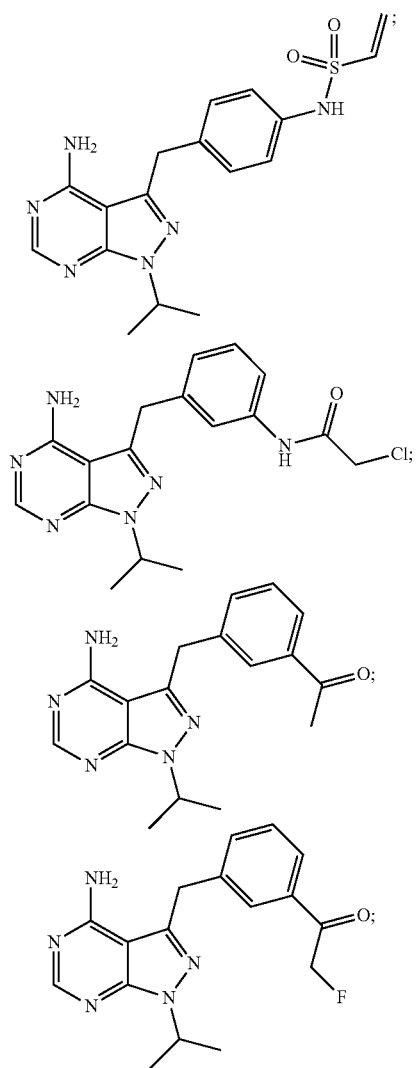
**272**

-continued

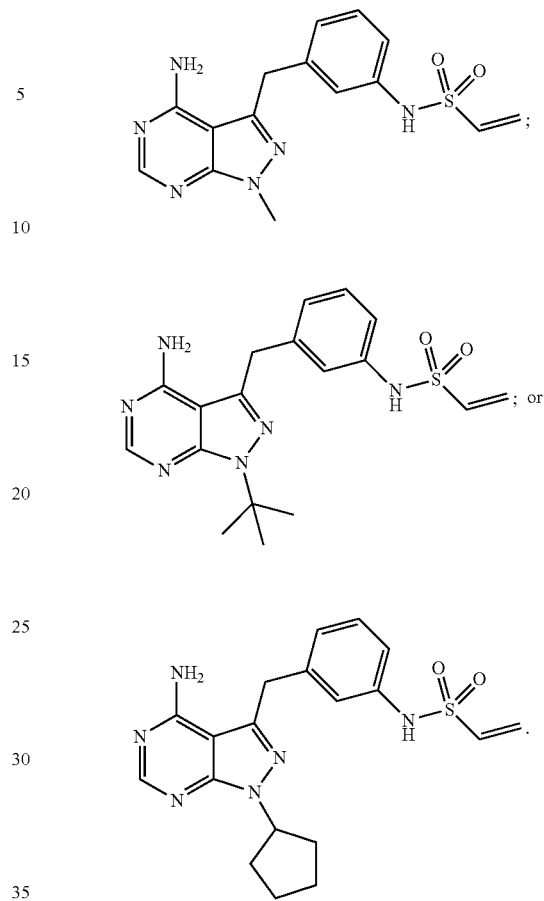


**273**

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**274**

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**12.** A pharmaceutical composition comprising a compound of claim 1 and a pharmaceutically acceptable excipient.

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